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# EXPERIMENTAL SKIN PAIN

## INDUCED BY INJECTION OF WATER-SOLUBLE SUBSTANCES IN HUMANS

OLOV LINDAHL

From Department of Physiology II (Head, Professor C. G. Bernhard)
and the Orthopedic Clinic (Head, Professor Sten Friberg)
Karolinska Institutet, Stockholm, Sweden

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# CHAPTER 1 REVIEW OF THE LITERATURE

## Experimental Investigations of Chemically Induced Pain

Previous investigations of experimental, chemically induced pain in humans are few and the results in part contradictory. Table 1 lists those authors who have reported — usually as incidental findings — the pain-producing effects of various aqueous solutions.

Elevation of the **osmotic pressure** above a certain level, it is reported, invariably gives rise to pain. According to different authors this level ranges between 2.8 and 5.7 times isotonic sodium chloride solution[2, 4, 13, 29, 32, 33, 37, 39, 73].

Hypotonic solutions also produce pain if the osmotic pressure is sufficiently reduced. The necessary reduction reportedly varies from 0.63 to 0.23 times isotonic sodium chloride solution[2, 4, 11, 13, 29, 37, 73]. Distilled water is said to induce severe pain[33, 37, 73].

Several authors have shown that **potassium** salts give rise to pain. The reported pain-producing concentration of $K^+$ varies, however, from 134 mN to 0.8 mN, the latter value being lower than the potassium concentration in plasma[2, 4, 11, 32, 33, 39, 73, 77 87].

*Fleckenstein*[26] stated that certain **intermediate metabolites** such as pyruvic acid, succinic acid and citric acid in neutral isotonic solution gave rise to pain in a concentration of approximately $10^{-2}$ to $10^{-3}$ g/g.

**Table 1** Data on previous investigations of chemically induced skin pain.

| AUTHOR | YEAR | MODE OF APPLICATION | PAIN-PRODUCING CONCENTRATION | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | ELEVATED OSMOTIC PRESSURE* | REDUCED OSMOTIC PRESSURE* | POTASSIUM ION mN | HYDROGEN ION pH | HISTAMINE G/ML | ACETYL-CHOLINE G/ML | SEROTONIN G/ML |
| *Grützner*[32] | 1894 | Brushing in wounds | 6.6 × | — | 134 | 1 N HCl | — | — | — |
| *Hacker*[33] | 1914 | Intracutaneous injection | 9.1 × | — | 161 | 0.01 N HCl | — | — | — |
| *Rodhe*[73] | 1921 | Intracutaneous injection | 5.7 × | 0.23 × | 134 | — | — | — | — |
| *Hecht*[37] | 1923 | Intracutaneous injection | 5.7 × | 0.28 × | — | — | — | — | — |
| *Bommer*[11] | 1924 | Intracutaneous injection | — | 0.5 × | 0.8 | — | — | — | — |
| *Braun*[13] | 1925 | Intracutaneous injection | 2.8 × | 0.63 × | — | — | — | — | — |
| *Hoff*[39] | 1926 | Intracutaneous injection | 5.6 × | 0.34 × | 161 | — | — | — | — |
| *Gaza*[29] | 1926 | Intracutaneous injection | 2.8 × | 0.28 × | — | 7.1 | — | — | — |
| *Rosenthal*[76] | 1939 | Intracutaneous injection | — | — | — | — | $0.5\times10^{-5}$ | $2.5\times10^{-4}$ | — |
| *Rosenthal*[77] | 1948 | Intracutaneous injection | — | — | 13 | — | $10^{-18}$ | $2\times10^{-9}$ | — |
| *Rosenthal*[75] | 1950 | Intracutaneous injection | — | — | — | — | $10^{-15}$ | — | — |
| *Armstrong*[2] | 1951 | Cantharidin blister lesions | 3.4 × | 0.34 × | 16 | 2.5 | $10^{-8}$ | $3\times10^{-5}$ | — |
| *Armstrong*[3] | 1952 | Cantharidin blister lesions | — | — | — | — | — | — | $10^{-8}$ |
| *Armstrong*[4] | 1953 | Cantharidin blister lesions | 5.7 × | 0.34 × | 16 | 3.0 | $10^{-8}$ | $10^{-4}$ | — |
| *Armstrong*[5] | 1957 | Cantharidin blister lesions | — | — | 16 | — | $10^{-5}$ | $10^{-5}$ | $10^{-9}$ |
| *Skouby*[87] | 1953 | Intracutaneous injection | — | — | 40 | — | $10^{-4}$ | No pain | — |

*In relation to isotonic with normal plasma

*Rosenthal et al.*[74, 75, 76, 77] demonstrated a pain-producing effect of **histamine** in a concentration as low as $10^{-18}$ g/g. *Skouby*[87] and *Armstrong et al.*[2, 4], were able to elicit pain with histamine only when the concentration exceeded $10^{-4}$ to $10^{-3}$ g/g.

*Armstrong et al.*[2] induced pain with **acetylcholine** at a concentration of $3 \cdot 10^{-5}$, whereas *Rosenthal et al*[76, 77] recorded no pain until the concentration reached $0.25 \cdot 10^{-3}$ to $2 \cdot 10^{-3}$ g/g, and *Skouby*[87] found no pain whatsoever with acetylcholine.

*Armstrong et al*[5] reported that **serotonin** produced pain in a concentration of $10^{-9}$ g/g or higher.

*Hacker*[33] elicited pain with dilute mineral **acids.** *Gaza & Brandi*[29] found that buffer solutions of pH 7.2 or lower were pain-producing. *Armstrong et al.*[2, 4] however, recorded pain only at pH 3 or lower.

The data obtained are thus, in part, contradictory; but above all they are merely qualitative. Moreover, the reported pain-producing concentrations of tested agents vary widely — in the case of histamine, for example, by as much as $10^{15}$ times. Frequently the reader is left in a state of perplexity as to the significance of various substances in the elicitation of pain.

### Chemical Methods for Inducing Skin Pain

Varying modes of application have been employed for eliciting pain by chemical means. Some authors have placed the test solution directly upon the skin or mucous membrane[1, 51]. This procedure requires strong solutions and the resulting responses are unreliable.

Hence it has been sought instead to apply the test solutions in direct contact with the tissues, particularly the naked nerve

terminals. *Armstrong et al.*[2] pricked the skin with a needle through the test solution. *Grützner*[32] made small skin incisions on the fingers of his subjects, then brushed the test solution into the wounds. *Rosenthal et al.*[76] removed a small piece of the outer epidermal layer with a razor, then dropped the solution on the defect. *Armstrong et al.*[2, 4] applied cantharidin to the skin overnight, then opened the resulting blister and dropped the test solution into the skin defect. It was possible to test a number of solutions on the same defect provided the area was rinsed with physiological salt solution and intervals of a minute or so allowed between the tests.

The most common procedure is probably to inject the test solution with fine needles intracutaneously or subcutaneously, or even intramuscularly. With subcutaneous injections the pain induced depends upon the point reached by the solution beneath the skin. The pain will be more intense in the vicinity of a nerve and also when the solution is near the epidermis, while deeper in the tissue the same injection will have a less painful effect. Hence the pain responses to solutions tested in this way show a wide range.

Intracutaneous injection seems to be the most frequently employed mode of application[11, 13, 26, 29, 33, 37, 39, 73, 75, 76, 77, 87]. Even this method has its drawbacks; for instance, the appreciable pain associated with application, and the difficulty of insuring a uniform injection rate and depth.

Intra-arterial injections have been tried experimentally only in animals[15, 16, 23, 62]. As in all animal experiments, it is here difficult or impossible to estimate the intensity of the pain; it must suffice to record reflex activity, muscle activity, vocalization, etc. There are only occasional instances of intra-arterial injections in humans, but these have yielded useful information[14, 35].

Although intramuscular injections in humans have been

reported[29, 58], the examiners have experienced difficulty in grading the pain intensity. Pain in muscle tissue is usually diffuse and cannot be readily defined.

### Methods of Evaluating Pain Intensity

Some investigators have been content to record whether or not pain has occurred, but in general it has been sought to grade the intensity of the pain as one-plus, two-plus, three-plus, etc. The numerals 1 to 4 have sometimes been substituted for the plus signs, or in turn have been replaced by definitive terms such as slight, moderate, severe, and very severe[7, 22, 41, 43, 49].

*Hardy, Wolff & Goodell*[34] in their extensive experimental investigations of pain induced by heat, introduced the term "just noticeable difference", abbreviated jnd. They showed that the range between scarcely perceptible and maximal pain comprised 21 jnd intervals. Two jnd's were called one dol. Trained subjects were able to estimate with some accuracy the pain in dols (pain units) that followed any specific thermal stimulus. The relevant scale had a range of zero to ten dols. It follows that trained subjects at least are well able to distinguish between varying degrees of pain.

*Armstrong et al.*[2, 4] employed graphic recording of the pain intensity. Their subjects squeezed a rubber balloon connected to a pen the movements of which were recorded on paper.

*Rosenthal et al.*[74] undertook statistical analyses to estimate the significance of their observations. The experiments conducted by most other authors have not been sufficiently numerous to permit statistical analysis.

Recent interest in evaluation of pain for the purpose of investigating the analgetic effects of morphine and similar drugs, has led to the development of reliable statistical methods in conjunction with double blind techniques. Although the aim

of the present investigation is somewhat different, the problem as well as the methods of evaluation are similar, whether it is a matter of measuring induced pain or of measuring spontaneous pain alleviated by analgesics. Investigations of this type have been conducted notably by *Beecher*[7, 88, 89], *Houde*[41, 83] and *Lasagna*[46, 47, 48, 49]. These authors concur on the following general principles for measurement of pain: (1) A double blind technique is essential; (2) the subjects should serve as their own controls unless very large numbers of experimentees are used; (3) expert statisticians should collaborate in statistical analyses of the observations.

The same authors also discuss the question of the proportionality of the scales when subjective criteria such as slight, moderate and severe pain are employed. Alternate use of parametric and non-parametric methods of statistical analysis has demonstrated that for comparison of different doses of the same analgesic, parametric methods (analysis of variance) are the most precise. The authors conclude, accordingly, that these subjective scales are virtually proportional, even though no conclusive evidence thereof can be secured.

The few previous investigations into experimental, chemically induced pain have not explored the subject with sufficient thoroughness, perhaps chiefly due to lack of uniform methods of pain measurement.

In the investigation reported here my aims have been

(1) to devise a chemical method of producing experimental skin pain and of measuring its intensity; and

(2) to determine systematically, with the use of that method, the pain-producing effects of various substances which occur in the organism and which are thought to be associated with pain.

# PART I

## METHOD FOR INDUCTION OF EXPERIMENTAL SKIN PAIN AND MEASUREMENT OF ITS INTENSITY

## CHAPTER 2 EXPERIMENTAL METHOD

### Subjects

The experiments were performed on healthy subjects of both sexes, ranging in age from 16 to 57 years and averaging thirty-six. Since the experiments in many instances were very painful, some of the subjects who took part in the first few tests subsequently dropped out. This resulted in some degree of selection, in that the remaining subjects were apparently more able to endure pain. The final group consisted of 20 subjects. Their ages varied between 16 and 57, the mean being 39 years.

### Apparatus

The test solutions were applied by a jet injector (Hygiscient, AB Kifa, Stockholm). With this instrument an electric vibration motor operates a piston at very high speed in a cylinder. The result is a pump action, which draws solution from the syringe to a nozzle, from which a jet 0.1 mm in diameter is ejected at high velocity and high pressure. It perforates the cutis and enters the subcutis. The injection procedure itself evokes little or no pain.

Trial injections of Evans blue in patients and of India ink in autopsy material showed that the jet entered the cutis and subcutis but did not penetrate the underlying fascia. The injection gave rise to an intracutaneous wheal 3 to 6 mm in dia-

meter and elevated 1 or 2 mm above the surrounding skin surface. In the subcutis was a diffuse conical injection area terminating at the fascia.

The duration of pain was recorded in seconds with a stop watch.

**Procedure**

With the aid of this jet injector 0.1 ml test solution was injected in each subject. In each run 16 such injections were given consecutively in each subject, eight different solutions being injected first on the outside of one upper arm, then on the contralateral arm. The pain caused by one injection was allowed to subside completely before the next injection was given. Neither the subjects nor the examiner (the present writer) knew which solutions the syringes contained, since the latter had been prepared for use as follows: Bottles containing eight different solutions were "shuffled" and placed in a row, and from them eight numbered syringes were filled in order. Not until the experiment had concluded were the solutions identified and annotated in the experimental records. The examiner thus knew which eight solutions were being tested but not which one each syringe contained. In this way a double blind technique was insured.

The subjects for their part estimated both the maximal pain intensity and the duration of pain. The estimated intensity was graded as follows:

**Intensity of pain**

| Grade | Units |
|---|---|
| NONE | **0** |
| None to very slight | 0.5 |
| VERY SLIGHT | **1.0** |
| Very slight to slight | 1.5 |
| SLIGHT | **2.0** |
| Slight to moderate | 2.5 |
| MODERATE | **3.0** |
| Moderate to severe | 3.5 |
| SEVERE | **4.0** |
| Severe to very severe | 4.5 |
| VERY SEVERE | **5.0** |

The subjects usually reported grades denoted by full units; the intermediate grades, though less frequently mentioned, were sometimes used to express minor differences in pain and sometimes, perhaps, as a sign of hesitation.

Eight different solutions were tested concurrently in each experiment, thus permitting comparison of their pain-producing effects. Fifteen to 21 subjects — usually 20 — took part in the tests.

## CHAPTER 3 STATISTICAL TREATMENT

Each of the test solutions was injected twice in each subject. Since the pain-producing effects were evaluated in 15—21 subjects, 30—42 values were obtained for the intensity, and a like number for the duration, of the pain induced by each solution. The pain values reported in the following are arithmetical means for these 30—42 injections.

Insofar as it was desired to compare directly the pain-producing effects of two solutions, the average pain response (intensity and duration) of each subject to those solutions was first calculated. The difference in the responses to the two solutions was than computed for each subject, after which the 15—21 values thus obtained were used for calculation of the mean ($m$), the standard deviation ( $\sigma$ ) and the standard error of the mean ( $\varepsilon$ ).

$$m = \frac{\Sigma x}{n} \qquad (1)$$

$$\sigma = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}} \qquad (2)$$

$$\varepsilon = \frac{\sigma}{\sqrt{n}} \qquad (3)$$

$n$ = number of observations

$x$ = differences in individual values (of pain intensity or duration)

The difference in pain responses (intensity and duration) to the solutions compared was checked for significance by means of the $t$ test.

$$t = \frac{m}{\varepsilon} \qquad (4)$$

The $p$ values were taken from conventional tables.

In all such comparisons the two solutions had been injected in the same subjects on the same occasions.

Regression analyses were undertaken in respect of hypertonic and hypotonic solutions, solutions with different potassium ion concentrations, and solutions with different hydrogen ion concentrations. These analyses were concerned with the relationship of the various concentrations of pain-producing agents to the intensity and duration of pain.

A record of experimental tests of varying hydrogen ion concentrations is exemplified below:

| SOLUTION | PAIN INTENSITY UNITS INJECTION NO. | | PAIN DURATION SECONDS INJECTION NO. | |
|---|---|---|---|---|
| | 1 | 2 | 1 | 2 |
| pH 5.6 | 1.0 | 2.0 | 7 | 34 |
| pH 3.6 | 3.0 | 4.0 | 36 | 12 |
| pH 5.1 | 3.5 | 2.0 | 12 | 8 |
| pH 3.2 | 4.5 | 4.5 | 20 | 22 |
| pH 4.1 | 4.0 | 5.0 | 22 | 20 |
| pH 6.2 | 1.0 | 1.0 | 7 | 5 |
| Sodium acetate | 1.0 | 0.5 | 8 | 2 |
| pH 4.6 | 4.0 | 4.0 | 16 | 19 |

This record pertains to a subject in whom each solution was injected twice, the two groups of figures for intensity and duration signifying the responses to two different injections.

For evaluation of any correlation that may have existed between the pain-producing effect and the hydrogen ion concentration, regression lines for both intensity and duration were plotted in respect of each subject. The equation for the regression line may be written as:

$$y = b \cdot x + a \qquad (5)$$

where $a$ is the distance of the line from the $x$ axis on the $y$ axis; $b$ is the tangent for the angle of the slope; $x$ is the hydrogen ion concentration ($K^+$ concentration, osmotic pressure); and $y$ represents the values for pain intensity (units) or pain duration (seconds). The values for $b$ and $a$ were computed from the following formulas:

$$b = \frac{\Sigma xy - \frac{\Sigma x \cdot \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} \qquad (6)$$

$$a = \frac{\Sigma y}{n} - b \cdot \frac{\Sigma x}{n} \qquad (7)$$

The arithmethical mean, standard deviation and standard error of the mean for the 20 $a$ and $b$ values were then calculated, in respect of pain intensity and duration, from formulas (1), (2) and (3).

The slope of the average regression line (mean of the $b$ values) was thereafter compared with that of the $x$ axis ($b = 0$), and the difference in slope (mean of the $b$ values) was examined for significance by means of the $t$ test.

A regression analysis was also undertaken in regard to the relation of intensity to duration for different types of pain-producing agents, the above formulas (6), (7), being used for computation of the $b$ and $a$ values. For checking the significance of the regression a formula differing from the above was used, namely:

$$s = \sqrt{\frac{1}{n-2} \cdot \left(u - \frac{v^2}{z}\right)} \quad (8)$$

$$\varepsilon_b = \frac{s}{\sqrt{z}} \quad (9)$$

The significance was determined by the $t$ test (formula 4), the number of degrees of freedom being $n - 2$.

$n$ = number of observations

$s$ = standard deviation about the regression line

$x$ = individual values of pain intensity (units)

$y$ = individual values of pain duration (seconds)

$$u = y^2 - \frac{(\Sigma y)^2}{n}$$

$$v = \Sigma xy - \frac{\Sigma x \cdot \Sigma y}{n}$$

$$z = \Sigma x^2 - \frac{(\Sigma x)^2}{n}$$

In a few instances analysis of covariance was employed to ascertain if the difference in the slopes of two regression lines was significant. For this purpose the following formulas were used:

$$s_{1+2} = \sqrt{\frac{(n_1 - 2) \cdot s_1^2 + (n_2 - 2) \cdot s_2^2}{n_1 + n_2 - 4}} \qquad (10)$$

$$\varepsilon_{b_1 - b_2} = s_{1+2} \cdot \sqrt{\frac{1}{z_1} + \frac{1}{z_2}} \qquad (11)$$

$$t = \frac{b_1 - b_2}{\varepsilon_{b_1 - b_2}} \qquad (12)$$

The significance wass determined by the $t$ test (formula 12). The number of degrees of freedom was here $n_1 + n_2 - 4$. The symbols have the same signification as before. Their subscript numbers denote that they are referable to the two lines which are to be compared.

The statistical section of this investigation was planned and executed in consultation with the Statistical Research Group, University of Stockholm.

CHAPTER 4

# BASAL PAIN ASSOCIATED WITH THE METHOD

In evaluation of the average pain responses to different test solutions, the "basal pain" associated with the method must be taken into account. On injection of isotonic sodium chloride solution in 20 subjects, variations occur not only in the individual responses to two injections but also in the average responses of different subjects (figure 1). In the latter instance the varying average responses might be attributable to differing sensitivity to pain, but in the case of two tests on a single subject some other explanation would be more plausible.

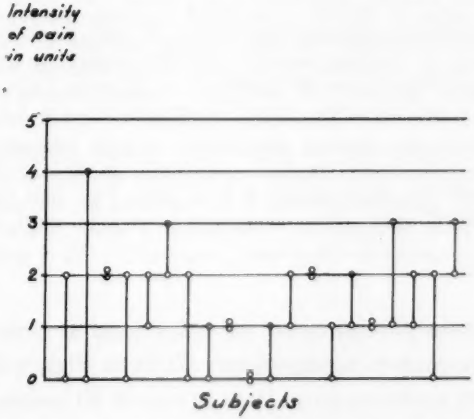



*Fig. 1* Variation of pain intensity in individual subjects with two similar injections of isotonic sodium chloride solution. Each point signifies one pain response, and a vertical line connects the two responses in each subject.

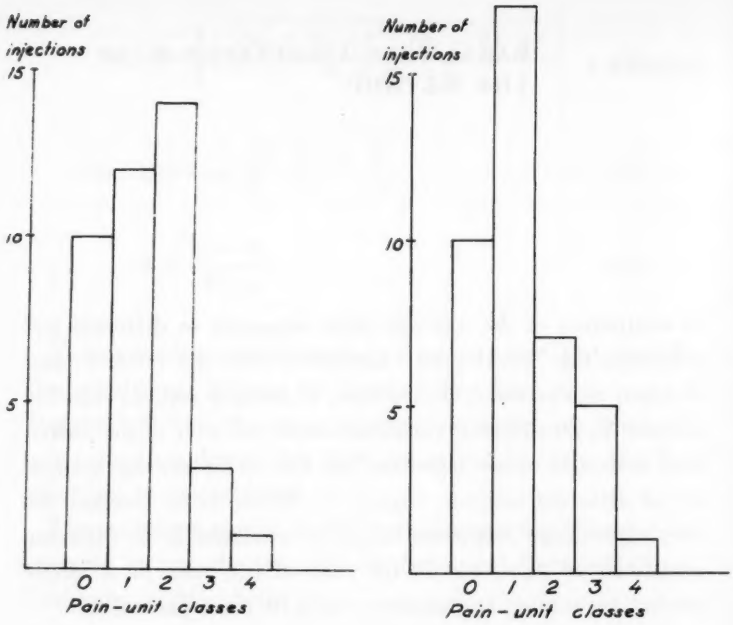
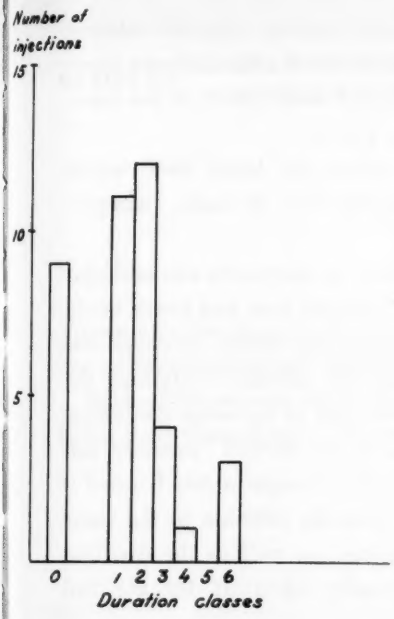



*Fig. 2* Histogram showing distribution of pain intensity following 40 injections of isotonic sodium chloride solution in 20 subjects. Average pain intensity for all injections is 1.33 units. Pain unit class 0 comprises 0 and 0.5 units; class 1 comprises 1.0 and 1.5 units, etc.

*Fig. 3* Histogram showing distribution of pain intensity following 40 injections of isotonic standard salt solution in 20 subjects. Average pain intensity for all injections is 1.26 units. Pain unit class 0 comprises 0 and 0.5 units; class 1 comprises 1.0 and 1.5 units, etc.

In many cases the injections were described as quite painless. In some cases they produced very slight or slight pain, and in three cases moderate pain. Only in one of 40 instances did an injection of isotonic sodium chloride solution give rise to severe pain (figure 1). The explanation may be that in general the injection trauma to the tissue is inappreciable and thus causes



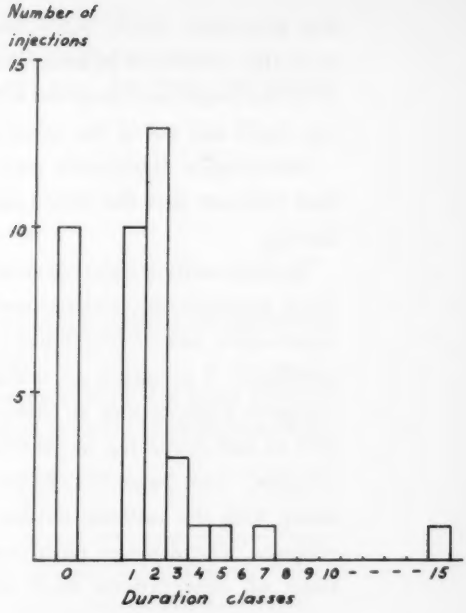




*Fig. 4* Histogram showing distribution of pain duration following 40 injections of isotonic sodium chloride solution in 20 subjects. Average pain duration for all injections is 8 seconds. Duration class 0 comprises 0 seconds; class 1 comprises 1—5 seconds; class 2, 6—10 seconds; class 3, 11—15 seconds; class 4, 16—20 seconds, etc.

*Fig. 5* Histogram showing distribution of pain duration following 40 injections of isotonic standard salt solution in 20 subjects. Average pain duration for all injections is 7 seconds. Duration class 0 comprises 0 seconds; class 1 comprises 1—5 seconds; class 2, 6—10 seconds; class 3, 11—15 seconds; class 4, 16—20 seconds, etc.

no pain, while in other cases the jet of fluid is more traumatizing and gives rise to a certain amount of pain due to the tissue injury.

On injection of solutions which are pain-producing per se it is essential, therefore, to take into account the basal pain associated with the method, i.e., the pain caused by the injec-

tion procedure itself. Since isotonic sodium chloride solution normally causes no pain in wounds or on subcutaneous injection, the basal pain must be regarded as an effect of the injection itself and not of the solution per se.

Statistically significant pain above the basal level would thus indicate that the tested solution was, in itself, pain-producing.

Isotonic sodium chloride solution, in contrast to extracellular fluid, contains only sodium and chloride ions and hence could conceivably have a slight pain-producing effect. To check this possibility I prepared an isotonic salt solution containing the ordinary body cations as chlorides and in the same concentration as that occurring in plasma. It was termed "standard salt solution" (see page 32). In one run of experiments I tested it along with the isotonic sodium chloride solution in the same subjects. The average pain responses as well as the distributions of observations were virtually identical for the two solutions.

| | NUMBER OF SUBJECTS | PAIN INTENSITY UNITS | PAIN DURATION SECONDS |
|---|---|---|---|
| Sodium chloride solution | 20 | 1.33 | 8 |
| Standard salt solution | 20 | 1.26 | 7 |

Cf. figures 2, 3, 4 and 5.

In some runs neither of these two solutions was used; the pain-producing effects of test solutions were instead evaluated in comparison with e.g. isotonic sodium bicarbonate or isotonic sodium acetate solution. Each of these two solutions, as will be reported later on, shows the same low average pain response as isotonic sodium chloride solution.

## CHAPTER 5 STANDARD DEVIATION AND REPRODUCIBILITY OF THE METHOD

To estimate the standard deviation of the method, the following run of experiments was conducted.

Each of 20 subjects received 16 injections, the same solution being used throughout — a neutral isotonic potassium-sodium

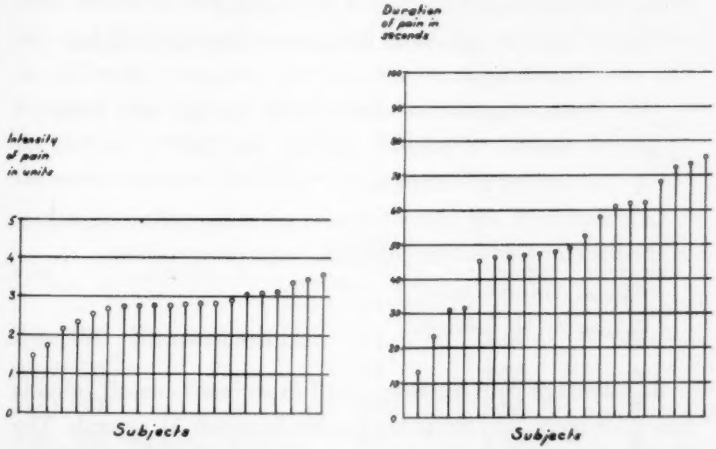



*Fig. 6* Variation of average pain intensity in 20 subjects who received a total of 320 injections of the same solution containing 77.7 mN potassium. Each point represents the mean of 16 injections in one subject. The responses are grouped according to their intensity.

*Fig. 7* Variation of average pain duration in 20 subjects who received a total of 320 injections of the same solution containing 77.7 mN potassium. Each point represents the mean of 16 injections in one subject. The responses are grouped according to their duration.

chloride solution containing 77.7 mN $K^+$. Such solution induces pain of moderate intensity.

The average pain response to the 16 injections was calculated for each subject. In the 20 subjects the mean intensity varied between 1.47 and 3.56 units, and the mean duration between 14 and 75 seconds (figures 6 and 7).

The variation in the values for any single subject — disregarding the varying sensitivity to pain of different subjects — can be computed statistically from the following formula:

$$\sigma = \sqrt{\frac{\Sigma ssq}{N-r}} \qquad (13)$$

$\sigma$ = standard deviation of the method
$ssq$ = sum of squares of deviations from the mean of one subject
$N$ = total number of observations (in this run, 320)
$r$ = number of subjects (in this run, 20)
$\Sigma$ = sum of all subjects

Calculation by the above formula gives the following values of the standard deviation for each single observation:

$\sigma_{intensity} = 0.64$ units
$\sigma_{duration} = 18$ seconds

The average pain intensity in all experiments on all subjects was 2.75 units, and the average pain duration 51 seconds. The coefficient of variation for a single observation was 23 per cent for intensity and 33 per cent for duration of pain. For a biologic method, these values must be considered satisfactory.

Since the subjects always received two injections of each solution, the coefficient of variation for each subject amounts to 16 per cent for intensity and 23 per cent for duration of pain. The fact that the pain-producing effects of solutions were

tested on 15—21 subjects constitutes a further substantial reduction of the experimental error.

In any comparison of the pain-producing effects of two solutions the methodologic error, however, is automatically taken into account and the analysis shows whether a difference is statistically significant or not.

Although the individual pain reaction to a given chemical stimulus varies considerably, as do the reactions of different individuals to the same painful stimulus, this method affords both numerical data on the pain intensity and a possibility of determining whether differences exist between test solutions, provided enough injections and subjects are used.

The reproducibility of the method is evident from its standard deviation and also from a comparison of the pain intensity and duration values obtained on testing of a given solution on different occasions. Isotonic sodium chloride solution and standard salt solution, which both represent the basal pain of the method, were tested on four different occasions largely on the same subjects. The following values emerged:

| DATE | SOLUTION | NUMBER OF SUBJECTS COMMON TO ALL EXPERIMENTS | PAIN DURATION SECONDS | PAIN INTENSITY UNITS |
|---|---|---|---|---|
| Febr./58 | Standard salt | 18 | **1.33** | **12** |
| April/58 | — „ — | 19 | **1.23** | **9** |
| Febr./59 | — „ — | 20 | **1.26** | **7** |
| Febr./59 | Sodium chloride | 20 | **1.33** | **8** |

It will be seen that the various determinations are in close accord.

The distribution of values in the series of 320 injections of the same solution (77.7 mN $K^+$) in 16 subjects was also studied. This distribution is illustrated in figures 8 and 9.

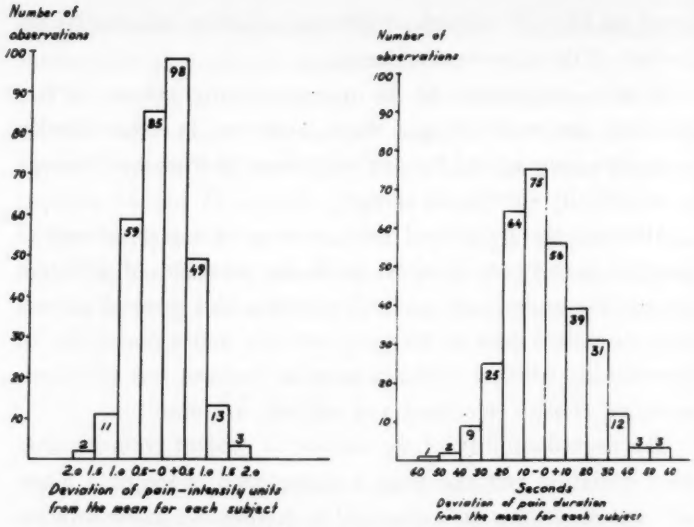



*Fig. 8* Histogram showing distribution of pain intensity in 20 subjects who received a total of 320 injections of the same solution containing 77.7 mN potassium. The classes are based on the mean of each subject, thus eliminating the variation of the mean for the individual subjects. Class range is 0.5 pain intensity units.

*Fig. 9* Histogram showing distribution of pain duration in 20 subjects who received a total of 320 injections of the same solution containing 77.7 mN potassium. The classes are based on the mean of each subject, thus eliminating the variation of the mean for the individual subjects. Class range is 10 seconds.

In this investigation the deviation from the mean for each subject was calculated in classes, each class range being half a unit for intensity and ten seconds for duration. The deviations from the mean for each subject were then totalled in the respective classes, thus securing the total number of observations in each class.

The observations both for intensity and for duration of pain showed a normal distribution.

# PART II

## PAIN-PRODUCING EFFECTS OF THE TEST SOLUTIONS

## CHAPTER 6 TEST SOLUTIONS

The solutions employed in this investigation were all aqueous and were prepared at the St. Görans Sjukhus pharmacy. Their osmotic pressures were ascertained by determining the reduction of the freezing point, using a method devised by *Bergström*[8]. The pH was determined by a pH meter (model pH M 4, Radiometer, Copenhagen). Acetylcholine and serotonin were prepared under aseptic precautions. Other solutions were sterilized in an autoclave (110° C for 30 minutes).

Solutions with the following composition were used:

**Solutions with Varying Osmotic Pressures**

| SOLUTION | SODIUM CHLORIDE mM | OSMOTIC PRESSURE MILLIOSMOL/L | pH |
|---|---|---|---|
| 1 × isotonic | 150 | 302 | 7.1 |
| 2 × „ | 300 | 610 | 7.1 |
| 3 × „ | 450 | 902 | 7.1 |
| 4 × „ | 600 | 1207 | 7.2 |
| 5 × „ | 750 | 1505 | 7.3 |
| 6 × „ | 900 | 1802 | 7.3 |
| ..... | | | |
| 0.8 × isotonic | 120 | 245 | 7.2 |
| 0.6 × „ | 90 | 180 | 7.2 |
| 0.4 × „ | 60 | 119 | 7.1 |
| 0.2 × „ | 30 | 59 | 7.1 |
| 0 × „ | 0 | 2 | 7.2 |

### Standard Salt Solution

To obtain a solution differing as little as possible from plasma in regard to mineral salts, the chlorides of sodium, potassium, calcium and magnesium were dissolved in distilled water in the following proportions: sodium 154, potassium 5, calcium 5 and magnesium 3 mN. This plasma concentration of cations had been reported by *Peters*[67]. The solution had an osmotic pressure of 316 milliosmol/1 and a pH of 7.3.

### Solutions with Varying Potassium Concentrations

These solutions were prepared by mixing the above standard salt solution (osmotic pressure 316 milliosmol/1) with 161 mN potassium chloride solution (osmotic pressure 318 milliosmol/1), the resulting solutions having the following potassium content:

| POTASSIUM ION CONCENTRATION mN | pH | |
|---|---|---|
| 5.0 | 7.0 | |
| 18.4 | 7.0 | |
| 31.8 | 6.9 | |
| 45.2 | 7.0 | |
| 58.6 | 7.1 | |
| 72.0 | 7.0 | |
| 85.4 | 7.0 | |
| 98.8 | 6.8 | |
| 77.7 | 6.9 | (Method test solution) |

### Isotonic Inorganic Salt Solutions

These consisted of the following salts dissolved in distilled water:

| SALT | CONCENTRATION mM | OSMOTIC PRESSURE MILLIOSMOL/L | pH |
|---|---|---|---|
| Sodium chloride | 150 | 302 | 7.1 |
| Magnesium chloride | 265 | 327 | 7.5 |
| Calcium chloride | 226 | 310 | 5.3 |
| Ammonium chloride | 150 | 312 | 6.7 |
| Sodium sulfate | 132 | 325 | 7.4 |
| Sodium bicarbonate | 175 | 315 | 8.3 |
| Sodium orthophosphate (mono — H) | 130 | 298 | 7.4 |

### Isotonic Solutions of Sodium Salts with Organic Acids

For these solutions the undermentioned salts were dissolved in distilled water:

| SALT | CONCENTRATION mM | OSMOTIC PRESSURE MILLIOSMOL/L | pH |
|---|---|---|---|
| Sodium citrate | 108 | 310 | 8.8 |
| Sodium lactate | 218 | 285 | 7.4 |
| Sodium acetate | 151 | 295 | 8.5 |
| Sodium succinate | 101 | 283 | 8.1 |
| Sodium pyruvate | 153 | 239 | 8.4 |
| Sodium acetate and | 100 | 305 | 8.0 |
| Sodium chloride | 51 | | |

The last solution had the same acetate ion content as the acetate buffers with which it was compared.

### Solutions of Organic Compounds

#### Creatine

Creatine 99 mM and sodium chloride 125 mM were dissolved in distilled water. The osmotic pressure was 302 milliosmol/l and the pH 7.4. The water-solubility of creatine does not suffice for an isotonic solution of creatine alone.

**Creatinine**

Creatinine 272 mM and sodium chloride 21 mM were dissolved in distilled water. The osmotic pressure was 298 milliosmol/l and the pH 6.5. The water-solubility of creatinine is not sufficient for preparation of an isotonic solution with that substance alone.

**Acetylcholine**

Acetylcholine chloride was dissolved in the aforementioned standard salt solution in concentrations of 5.1 mM or $10^{-3}$ g/ml (pH 7.0) and 0.5 mM or $10^{-4}$ g/ml (pH 7.0). The solutions were prepared under aseptic precautions immediately prior to the injections.

**Serotonin**

Serotonin was dissolved in the standard salt solution in concentrations of 5.7 mM or $10^{-3}$ g/ml (pH 7.5), 0.6 mM or $10^{-4}$ g/ml (pH 7.5) and 0.06 mM or $10^{-5}$ g/ml (pH 7.6). The solutions were prepared under aseptic precautions immediately before the injections.

**Histamine**

Histamine chloride was dissolved in the standard salt solution in concentrations of 5.6 mM or $10^{-3}$ g/ml (pH 5.8), 0.6 mM or $10^{-4}$ g/ml (pH 6.0), 0.06 mM or $10^{-5}$ g/ml (pH 6.5) and 0.006 mM or $10^{-6}$ g/ml (pH 7.1).

**Solutions with Varying Hydrogen Ion Concentrations**

**Series with different buffers and pH from 1.1 to 7.2**

Measured **pH 1.1:** 48.5 ml 0.2 N HCl — 25 ml 0.2 N KCl — 26.5 ml distilled water. Osmotic pressure 256 milliosmol/l.

Measured **pH 3.2:** 97.5 ml 0.1 N acetic acid — 2.5 ml 0.1 N sodium acetate — 0.36 g sodium chloride. Osmotic pressure 390 milliosmol/l.

Measured **pH 5.1:** 29.0 ml 0.1 N acetic acid — 71.0 ml 0.1 N sodium acetate — 0.19 g sodium chloride. Osmotic pressure 341 milliosmol/l.

Measured **pH 7.2:** 29.6 ml 0.1 N NaOH — 50 ml 0.1 M $KH_2PO_4$ — 20.4 ml distilled water — 0.54 g sodium chloride. Osmotic pressure 220 milliosmol/l.

**Series with acetate buffers and pH from 3.2 to 6.2**

| MEASURED pH | 0.1 N ACETIC ACID ML | 0.1 N SODIUM ACETATE ML | SODIUM CHLORIDE G | OSMOTIC PRESSURE MILLIOSMOL/L |
|---|---|---|---|---|
| 3.2 | 97.5 | 2.5 | 0.36 | 390 |
| 3.6 | 92.5 | 7.5 | 0.31 | 330 |
| 4.1 | 80.0 | 20.0 | 0.29 | 333 |
| 4.6 | 57.5 | 42.5 | 0.26 | 341 |
| 5.1 | 29.0 | 71.0 | 0.19 | 341 |
| 5.6 | 12.0 | 88.0 | 0.15 | 352 |
| 6.2 | 4.0 | 96.0 | 0.13 | 349 |

**Series with ammonia buffers and pH from 7.6 to 10.6**

| MEASURED pH | 0.1 N $H_4NCl$ ML | 0.1 N $H_3N$ ML | SODIUM CHLORIDE G | OSMOTIC PRESSURE MILLIOSMOL/L |
|---|---|---|---|---|
| 7.6 | 98.0 | 2.0 | 0.14 | 364 |
| 8.1 | 93.5 | 6.5 | 0.16 | 339 |
| 8.6 | 81.5 | 18.5 | 0.17 | 356 |
| 9.1 | 58.0 | 42.0 | 0.18 | 320 |
| 9.6 | 31.0 | 69.0 | 0.26 | 328 |
| 10.1 | 12.5 | 87.5 | 0.26 | 324 |
| 10.6 | 4.5 | 95.5 | 0.27 | 326 |

## Data on the Substances Used

**Substances conforming with the requirements of the Swedish Pharmacopoeia Edition XI**

Acetic acid
Ammonium chloride
Ammonium hydroxide
Calcium chloride ($CaCl_2 \cdot 2\ H_2O$)
Distilled water
Histamine chloride (di-chloride)
Hydrochloric acid
Lactic acid
Potassium chloride
Potassium orthophosphate ($K_2HPO_4$)
Sodium acetate ($NaC_2H_3O_2$)
Sodium bicarbonate
Sodium chloride
Sodium citrate ($Na_3C_6H_5O_7 \cdot 2\ H_2O$)
Sodium hydroxide
Sodium sulfate ($Na_2SO_4 \cdot 10\ H_2O$)

**Substances obtained from E. Merck, Darmstadt.**

Creatine
Creatinine
Magnesium chloride
Sodium orthophosphate ($Na_2HPO_4$)
Sodium pyruvate ($NaC_3H_3O_3$)
Sodium succinate ($Na_2C_4H_4O_8 \cdot 6\ H_2O$)

**Substances obtained from F. Hoffman-La Roche & Co. A.G., Basle.**

Acetylcholine chloride

**Substances obtained from Sandoz A.G., Basle.**

Serotonin (5-hydroxytryptamine)

Sodium lactate ($NaC_3H_5O_3$) was prepared from lactic acid and sodium hydroxide.

## CHAPTER 7 OSMOTIC PRESSURE

### Hypertonic Solutions

For determination of the pain-producing effect of an increased osmotic pressure, six neutral aqueous solutions of sodium chloride were tested in one run of experiments. The osmotic pressures of these solutions ranged from isotonic to six times the osmotic pressure of plasma. All six of them were injected on the same occasion, each subject receiving two injections of each solution. The experiment comprised 15 subjects. The reported average intensities and durations are the arithmetical means of 30 injections.

The results are set forth in table 2 and figure 10.

**Table 2** Average pain responses to neutral hypertonic sodium chloride solutions tested on 15 subjects. Each solution injected 30 times. All solutions tested concurrently.

| SOLUTION | CONCENTRATION OF NaCl mM | OSMOTIC PRESSURE MILLIOSMOL/L | PAIN INTENSITY UNITS | PAIN DURATION SECONDS |
|---|---|---|---|---|
| 1 × isotonic | 150 | 302 | **1.98** | **23** |
| 2 × „ | 300 | 610 | **2.98** | **38** |
| 3 × „ | 450 | 902 | **3.10** | **48** |
| 4 × „ | 600 | 1207 | **3.02** | **61** |
| 5 × „ | 750 | 1505 | **3.33** | **66** |
| 6 × „ | 900 | 1802 | **3.80** | **89** |

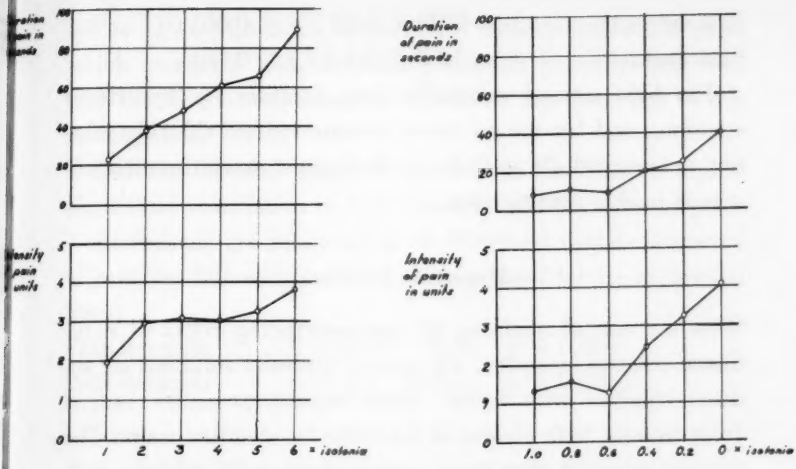



*Fig. 10* Diagram showing relation of pain — measured as intensity and duration — to elevated osmotic pressure. Each point on the curves represents the mean of 30 injections in 15 subjects. The test solutions were of sodium chloride with an osmotic pressure ranging from isotonic to six times that of the body.

*Fig. 11* Diagram showing relation of pain — measured as intensity and duration — to reduced osmotic pressure. Each point on the curves represents the mean of 40 injections in 20 subjects. The test solutions were of sodium chloride with an osmotic pressure ranging from zero to isotonic with that of the body.

It will be seen that elevation of the osmotic pressure above the normal gave rise to pain, which increased proportionately with the osmotic pressure. Analysis revealed a statistically significant correlation between osmotic pressure and both intensity and duration of pain.

The following *b* values were obtained for the regression lines for the correlation between increased osmotic pressure and pain:

Pain intensity $b = 0.29 \mp 0.03$ $(p < 0.001)$
Pain duration $b = 12.2 \mp 2.1$ $(p < 0.001)$

The pain was of unusually long duration for hypertonic solutions, and for the six times isotonic sodium chloride solution it averaged 89 seconds — the longest mean duration observed in this investigation.

### Hypotonic Solutions

With the aim of studying the pain-producing effect of a reduced osmotic pressure, six neutral aqueous solutions of sodium chloride were tested. Their osmotic pressures ranged from isotonic with plasma to the value for distilled water. The various solutions were tested concurrently on 20 subjects, each solution being injected 40 times. The results are presented in table 3 and figure 11.

**Table 3** Average pain response to neutral hypotonic sodium chloride solutions tested on 20 subjects. Each solution injected 40 times. All solutions tested concurrently.

| SOLUTION | CONCENTRATION OF NaCl mM | OSMOTIC PRESSURE MILLIOSMOL/L | PAIN INTENSITY UNITS | PAIN DURATION SECONDS |
|---|---|---|---|---|
| 1 × isotonic | 150 | 302 | **1.33** | **8** |
| 0.8 × „ | 120 | 245 | **1.58** | **11** |
| 0.6 × „ | 90 | 180 | **1.28** | **9** |
| 0.4 × „ | 60 | 119 | **2.45** | **20** |
| 0.2 × „ | 30 | 59 | **3.29** | **25** |
| 0 × „ | 0 | 2 | **4.05** | **40** |

It will be seen that a moderate decrease of the osmotic pressure did not induce pain. Not until the osmotic pressure

fell to 119 milliosmol/l or lower did the solutions cause pain, which increased with decreasing osmotic pressure and was, with distilled water, almost as severe as any recorded in this investigation.

Reduced osmotic pressure showed, on analysis, a highly significant correlation to both intensity and duration of pain.

As regards the relationship of decreased osmotic pressure to pain, the following *b* values were obtained for the regression lines:

| | |
|---|---|
| Pain intensity | $b = 0.56 \mp 0.03 \quad (p < 0.001)$ |
| Pain duration | $b = 6.1 \mp 0.6 \quad (p < 0.001)$ |

### Relationship of Pain Intensity to Pain Duration

A study of figures 10 and 11 reveals that the intensity and duration of pain showed largely parallel variations with changes in degree of the painful stimulus. Calculation of the regression lines for the correlation between intensity and duration discloses not only that there is a highly significant regression for both hypertonic and hypotonic solutions ($p < 0.001$) but also that the slope of the regression line differs for the two types of solutions (figure 16, page 60), the difference being highly significant ($p < 0.001$).

## CHAPTER 8 | INORGANIC IONS

Isotonic neutral solutions of the inorganic ions commonly occurring in the blood were tested in the form of sodium salts or chlorides. Since the solutions were not tested concurrently but in different runs of experiments, they cannot be directly compared. The pain-producing effects are set forth in table 4, which shows that, for each solution, the average pain intensity was very slight to slight and the duration short.

**Table 4** Average pain responses to solutions of various inorganic ions tested on 20 subjects, though not all concurrently. Each solution injected 40 times. The solutions were isotonic and their compositions are reported on page 32. Those tested concurrently have identical serial numbers.

| SALT | SERIES NO. | PAIN INTENSITY UNITS | PAIN DURATION SECONDS |
|---|---|---|---|
| $NaCl$ | 7 | **1.33** | **8** |
| $MgCl_2$ | 7 | **1.48** | **11** |
| $CaCl_2$ | 8 | **1.90** | **11** |
| $Na_2SO_4$ | 8 | **1.90** | **15** |
| $H_4NCl$ | 9 | **2.05** | **24** |
| $NaHCO_3$ | 9 | **1.46** | **14** |
| $Na_2HPO_4$ | 9 | **1.46** | **11** |

Only magnesium chloride solution was tested concurrently with isotonic sodium chloride solution (basal pain of the method). No difference was found in the pain-producing effects of these solutions.

Since the other solutions were not tested concurrently with isotonic sodium chloride or standard salt solution, no accurate estimate can be made of their pain-producing effects. The pain responses to sodium bicarbonate and sodium phosphate were numerically at the same level as isotonic sodium chloride solution, and the same subjects took part in the two experiments; hence, those two solutions as well may be considered equivalent to the basal pain level.

Comparison of sodium acetate (practically equivalent to the basal pain) with calcium chloride and with sodium sulphate revealed no significant difference. Comparison of sodium bicarbonate and ammonium chloride showed an almost significant difference with respect to pain intensity ($0.01 < p < 0.05$).

**Potassium Ion**

Previous investigators have attributed marked pain-inducing properties to this ion, in contrast to the aforementioned ions. This led me to test $K^+$ in eight different concentrations ranging from 5.0 mN — which is the normal concentration in plasma — to 98.8 mN. The test solutions were neutral aqueous ones, the potassium concentration being varied by admixture of isotonic potassium chloride solution with standard salt solution in varying proportions. The pain-producing effects of these solutions are evident from table 5 and figure 12, which show that the pain response rose in direct relation to increasing potassium ion concentration.

Regression analysis disclosed a highly significant correlation between concentration and both intensity and duration of pain ($p < 0.001$).

**Table 5** Average pain responses to neutral isotonic solutions in which the potassium content ranged from 5.0 to 98.8 mN. Each solution tested on 20 subjects and injected 40 times. All solutions tested concurrently.

| POTASSIUM ION CONCENTRATION mM | PAIN INTENSITY UNITS | PAIN DURATION SECONDS |
|---|---|---|
| 5.0 | 1.23 | 9 |
| 18.4 | 1.54 | 12 |
| 31.8 | 1.78 | 12 |
| 45.2 | 1.95 | 17 |
| 58.6 | 2.05 | 24 |
| 72.0 | 2.53 | 25 |
| 85.4 | 2.49 | 27 |
| 98.8 | 2.85 | 41 |

The following *b* values were obtained for the regression lines in respect of the correlation between elevated potassium ion concentration and pain:

Pain intensity $b = 0.22 \mp 0.02$ $(p < 0.001)$

Pain duration $b = 4.1 \mp 0.8$ $(p < 0.001)$

Comparison of standard salt solution ($K^+$ content 5 mN) and the solution with a $K^+$ content of 18.4 mN showed no significant difference. On the other hand, a solution containing 31.8 mN potassium, when compared with standard salt solution, showed a significant difference for pain intensity ($0.001 < p < 0.01$) and an almost significant difference for pain duration ($0.01 < p < 0.05$). At a potassium concentration of 45.2 mN the difference was significant in respect to both the intensity and the duration of pain. However, the pain induced was slight and its duration short. For the solution with the highest potassium concentration (98.8 mN) the average pain

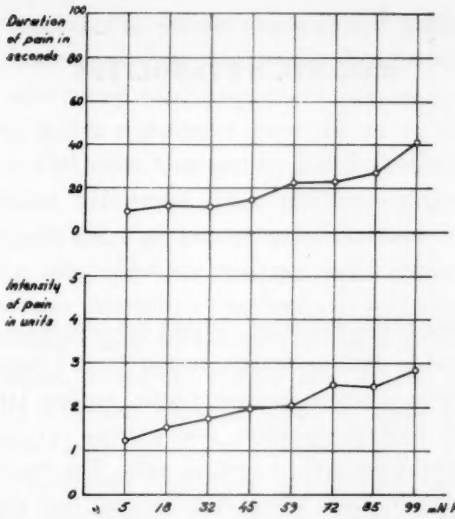



*Fig. 12* Diagram showing relation of pain — measured as intensity and duration — to the potassium ion concentration. Each point on the curves represents the mean of 40 injections in 20 subjects. The test solutions were neutral and isotonic, with a potassium ion content ranging from 5 to 99 mN.

was still less than moderate, even though the potassium content exceeded that in plasma by approximately 20 times.

There was here, just as in the case of solutions with differing osmotic pressures, a correlation between intensity and duration of pain. Calculation of the regression line for this correlation showed a highly significant regression ($p < 0.001$). See also figure 16, page 60.

## CHAPTER 9 ORGANIC METABOLITES

Pain-inducing properties have, during the course of the years, been ascribed to various organic metabolites. I have investigated creatinine, creatine, sodium citrate, sodium lactate, sodium acetate, sodium succinate, and sodium pyruvate in isotonic concentrations and as neutral salts. The results are set forth in table 6, from which it is evident that all of these solutions elicited pain responses of both low intensity and short duration.

**Table 6** Average pain response to solutions of various organic metabolites. The solutions were isotonic and their compositions are reported on page 33. Those tested concurrently have identical serial numbers. Each subject received two injections.

| METABOLITE | NUMBER OF SUBJECTS | SERIES NO. | PAIN INTENSITY UNITS | PAIN DURATION SECONDS |
|---|---|---|---|---|
| Creatinine | 20 | 10 | 1.14 | 6 |
| Creatine | 20 | 10 | 0.96 | 8 |
| Sodium citrate | 20 | 10 | 1.94 | 32 |
| Sodium lactate | 20 | 8 | 1.68 | 14 |
| Sodium acetate | 20 | 8 | 1.53 | 14 |
| Sodium succinate | 15 | 3 | 2.40 | 35 |
| Sodium pyruvate | 15 | 3 | 2.30 | 31 |

The pain responses to sodium succinate and sodium pyruvate appear to have been somewhat greater than the others. Those solutions were tested concurrently with isotonic sodium chloride, which in this experiment gave rise to an average pain intensity of 1.94 units. Comparison with that solution revealed no significant difference. This particular experiment comprised subjects with high average pain responses.

Since the other solutions were not tested concurrently with isotonic sodium chloride or standard salt solution, their pain-producing effects cannot be estimated with any accuracy. The pain responses to sodium acetate and sodium lactate were numerically very low, however; hence it seems unlikely that these solutions give rise to any significant pain.

In one experiment creatinine, creatine and sodium citrate were tested concurrently. No solution representing the basal pain of the method was included in this run. The pain responses to creatinine and creatine were, numerically, exceptionally low and differed significantly from the response to sodium citrate ($p < 0.001$ both for intensity and duration). Compared with creatinine, therefore, sodium citrate had a pain-producing effect.

## CHAPTER 10 HISTAMINE, SEROTONIN AND ACETYLCHOLINE

Each of these substances was added to standard salt solution (see page 32) in concentrations of $10^{-6}$ to $10^{-3}$ g/ml. They were tested in the usual way with 40 injections of each solution in a total of 20 subjects. Not all of them were tested concurrently, however, there being several runs of experiments. The results are shown in table 7.

**Table 7** Average pain responses to solutions of histamine, acetylcholine and serotonin in different concentrations. Each solution tested on 20 subjects and injected 40 times. Those tested concurrently have identical serial numbers.

| SOLUTION CONCENTRATION IN G/ML | SERIES NO. | PAIN INTENSITY UNITS | PAIN DURATION SECONDS |
|---|---|---|---|
| Histamine | | | |
| $10^{-6}$ | 9 | 1.43 | 12 |
| $10^{-5}$ | 9 | 1.50 | 11 |
| $10^{-4}$ | 8 | 2.29 | 15 |
| $10^{-3}$ | 8 | 2.49 | 18 |
| Serotonin | | | |
| $10^{-5}$ | 9 | 1.77 | 14 |
| $10^{-4}$ | 9 | 2.06 | 14 |
| $10^{-3}$ | 9 | 2.12 | 13 |
| Acetylcholine | | | |
| $10^{-4}$ | 8 | 1.61 | 11 |
| $10^{-3}$ | 8 | 2.01 | 16 |

These substances, even in relatively high concentrations, had little or no pain-producing effect. A noteworthy finding is that with the above solutions, histamine included, itching rarely arose; no more often, in fact, than with any other test solutions.

**Histamine** was tested in two different runs, the concentrations of $10^{-5}$ and $10^{-6}$ g/ml concurrently with isotonic sodium chloride solution. In these concentrations it produced no pain responses diverging from the basal pain. The concentrations of $10^{-3}$ and $10^{-4}$ g/ml were tested concurrently with isotonic sodium acetate solution, whose pain-producing effect is just as low as the basal pain. On comparison with that solution, histamine in each of these two concentrations had a significant pain-producing effect ($0.001 < p < 0.01$), though the average pain was only slight to moderate. In one subject histamine was also tested in a concentration of $10^{-2}$ g/ml. The pain responses to two injections were 1.0 and 3.0 units respectively; i.e., they were exceedingly moderate notwithstanding the fact that the concentration was high enough to give rise to general symptoms.

**Serotonin** was tested in concentrations of $10^{-3}$, $10^{-4}$ and $10^{-5}$ g/ml concurrently with isotonic sodium bicarbonate solution, whose pain-producing effect is no higher than the basal pain. In the concentration of $10^{-5}$ g/ml, serotonin did not differ in pain-producing effect from the comparative solution. At a concentration of $10^{-4}$ g/ml the difference in pain intensity was almost significant ($0.01 < p < 0.05$), and in a concentration of $10^{-3}$ g/ml the pain-producing effect was significant ($0.001 < p < 0.01$), though the average pain was slight and of very short duration.

**Acetylcholine** was tested in concentrations of $10^{-5}$ and $10^{-4}$ g/ml concurrently with isotonic sodium acetate solution. No difference in pain intensity between the latter solution and acetylcholine in the concentration of $10^{-4}$ g/ml was found. At

$10^{-3}$ g/ml the difference was almost significant ($0.01 < p < 0.05$); furthermore, the average pain induced was slight and of short duration.

## CHAPTER 11 | HYDROGEN ION CONCENTRATION

Since it had been observed quite early in this investigation that solutions with elevated hydrogen ion concentrations had marked pain-producing effects, solutions with varying hydrogen ion concentrations were tested more extensively than other factors.

Alkaline buffer solutions were subjected to one run and acid buffer solutions to two runs of experiments.

### Alkaline Buffer Solutions

The pain-producing effects of these solutions are presented in table 8 and figure 13, which show that the effects were quite moderate and that they increased somewhat with rising hydroxyl ion concentrations.

**Table 8** Average pain responses to alkaline ammonia buffer solutions tested on 21 subjects. Each solution injected 42 times. All solutions tested concurrently.

| pH | PAIN INTENSITY UNITS | PAIN DURATION SECONDS |
|---|---|---|
| 7.6 | 1.81 | 21 |
| 8.1 | 1.98 | 21 |
| 8.6 | 2.43 | 32 |
| 9.1 | 2.61 | 41 |
| 9.6 | 2.57 | 46 |
| 10.1 | 2.83 | 42 |
| 10.6 | 2.74 | 46 |

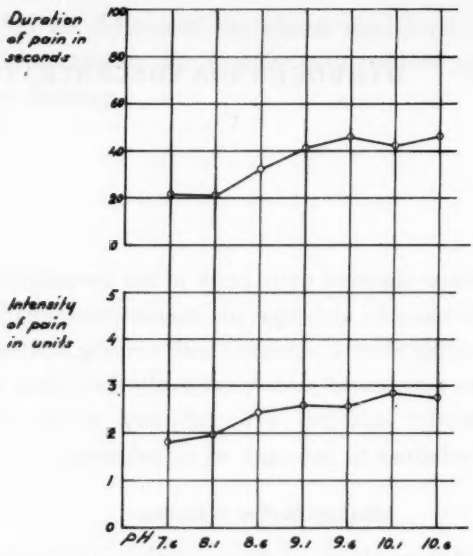



*Fig. 13* Diagram showing relation of pain — measured as intensity and duration — to the hydroxyl ion concentration. Each point on the curves represents the mean of 42 injections in 21 subjects. The buffer solutions were ammonia buffers.

In evaluating the pain responses to alkaline buffer solutions, it must be borne in mind that the ammonium ion present in those solutions has, in itself, a certain pain-producing effect. Isotonic ammonium chloride solution, tested on 20 subjects with 40 injections, produced an average pain response of 2.05 intensity units and 24 seconds duration. Though the above solution was not tested concurrently with the alkaline ammonium buffers, 18 of the subjects took part in both experiments. If we compare, in these 18 subjects, the pain-producing effects of isotonic ammonium chloride on the one hand, and those of alkaline buffer solutions on the other, the difference is not significant until the pH reaches a level considerably higher than that normal for the blood. See table 9.

### Acid Buffer Solutions

The pain-producing effects of these solutions are shown in tables 10 and 11 together with figures 14 and 15.

**Table 9** Difference in pain intensity between isotonic ammonium chloride on the one hand and alkaline ammonia buffer solutions on the other.

| pH OF BUFFER SOLUTIONS | DIFFERENCE IN PAIN INTENSITY ON COMPARISON WITH $H_4NCl$ | *P* VALUE OF DIFFERENCE |
|---|---|---|
| 7.6 | None | — |
| 8.1 | None | — |
| 8.6 | Almost significant | $0.01 < p < 0.05$ |
| 9.1 | Significant | $0.001 < p < 0.01$ |
| 9.6 | Almost significant | $0.01 < p < 0.05$ |
| 10.1 | Highly significant | $p < 0.001$ |
| 10.6 | Highly significant | $p < 0.001$ |

**Table 10** Average pain responses to acid buffer solutions tested on 16 subjects. Each solution injected 32 times. All solutions tested concurrently.

| pH | PAIN INTENSITY UNITS | PAIN DURATION SECONDS |
|---|---|---|
| 7.2 Phosphate buffer | **1.75** | **19** |
| 5.1 Acetate buffer | **3.11** | **26** |
| 3.2 Acetate buffer | **4.05** | **37** |
| 1.1 KCl-HCl buffer | **3.84** | **58** |

**Table 11** Average pain responses to acid acetate buffer solutions tested on 20 subjects. Each solution injected 40 times. All solutions tested concurrently.

| pH | PAIN INTENSITY UNITS | PAIN DURATION SECONDS |
|---|---|---|
| 6.2 | 1.81 | 10 |
| 5.6 | 2.51 | 14 |
| 5.1 | 3.34 | 12 |
| 4.6 | 3.54 | 12 |
| 4.1 | 3.86 | 14 |
| 3.6 | 3.90 | 17 |
| 3.2 | 4.18 | 19 |

It will be seen that they were pronounced, especially in respect to intensity, and rose with increasing hydrogen ion concentrations. On the whole, the effects produced in the two runs were equivalent.

Regression analysis of the relationship of pain responses to the logarithmic values for hydrogen ion concentrations discloses a significant correlation both for intensity and for duration in each of the two runs of experiments. The following *b* values were obtained for the regression lines for the correlation between pain and hydrogen ion concentration in the runs at pH 1.1 to 7.2:

Pain intensity $b = 0.74 \mp 0.06$ $(p < 0.001)$
Pain duration $b = 13.5 \mp 2.4$ $(p < 0.001)$

For the runs with acetate buffer solutions alone, the following values were obtained:

Pain intensity $b = 0.37 \mp 0.03$ $(p < 0.001)$
Pain duration $b = 1.3 \mp 0.4$ $(0.001 < p < 0.01)$

These buffer solutions contained acetate ions as well as hydrogen ions. Acetate ions were tested separately in isotonic

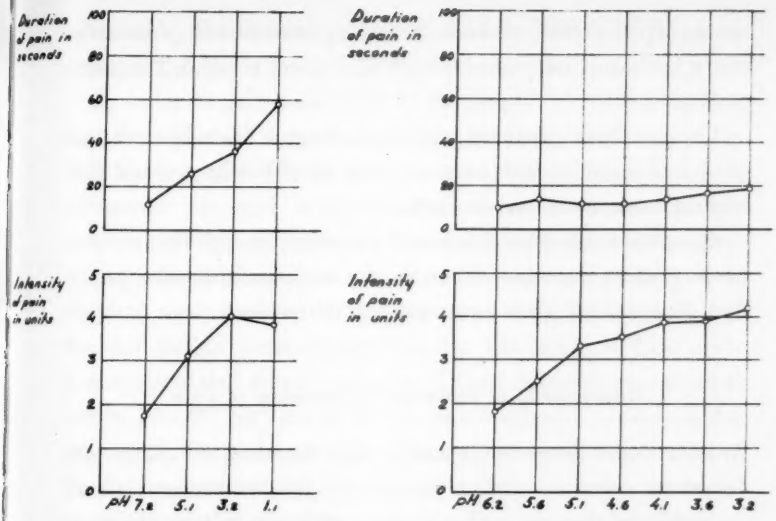



*Fig. 14* Diagram showing relation af pain — measured as intensity and duration — to the hydrogen ion concentration. Each point on the curves represents the mean of 32 injections in 16 subjects. The buffer solutions were of varying composition (see table 10).

*Fig. 15* Diagram showing relation of pain — measured as intensity and duration — to the hydrogen ion concentration. Each point on the curves represents the mean of 40 injections in 20 subjects. The buffer solutions were acetate buffers.

solution in the form of sodium acetate. Since this solution produced no pain that deviated from the basal pain of the method (see Chapter 9, page 46), it seems evident that the hydrogen ions constituted the pain-producing factor.

In the run of experiments with acid acetate buffer solutions (table 11) I tested concurrently with those solutions, an isotonic solution containing sodium acetate with the same concentration of acetate ions as that in the buffers (100 mN). Comparison of this solution with the buffer solution of pH 6.2 revealed a highly significant difference in pain intensity re-

sponse ($p < 0.001$); hence it can be stated with assurance that a hydrogen ion concentration equivalent to pH 6.2 is pain-producing.

The pain then mounted with the hydrogen ion concentration of the injected buffer solution and, at pH 3.2, reached the highest intensity values recorded.

The pain evoked by these acid solutions, though severe, was of very short duration; even for the most painful solution, it had disappeared after an average of 19 seconds.

### Relationship of Intensity to Duration of Pain

A correlation between intensity and duration of pain was found for solutions with varying hydrogen ion concentrations, as had been the case with solutions differing in osmotic pressure and in potassium ion concentration. Calculation of the regression lines for this correlation in respect of alkaline buffer solutions and acetate buffers showed a highly significant regression in each instance ($p < 0.001$). In figure 16 (page 60) it will be seen that the regression was far less for the acid than for the alkaline solutions. The difference in the slopes of the regression lines was highly significant ($p < 0.001$).

### Alteration of pH on Mixture of Buffer Solutions with Serum or Fluid Resembling Extracellular Fluid

On intracutaneous injection of a solution containing e.g. potassium ions, some time will elapse before those ions have been dissipated by the blood circulation and by diffusion, and the potassium concentration in the tissue has returned to normal. A similar mechanism may be expected on injection of buffer solution containing a high concentration of hydrogen ions, though the hydrogen ion concentration may be more swiftly

affected by the buffering properties of the body and the extracellular fluid.

In order to gain some idea of the magnitude of this buffering, I conducted several experiments. Four small pieces of skin were removed from autopsy cases, then denuded of subcutaneous fat and weighed. Into these specimens isotonic sodium chloride solution was injected by the jet injector, producing wheals throughout the skin. The specimens were then weighed again and the total water content, the fat content, and the dry weight were determined. On the basis of these data it was calculated that the amount of fluid taken up was equivalent to 70—80 per cent of the amount originally present in the specimens. On the assumption that some of the intracellular fluid does not mix with the injected solution, it may be roughly estimated that the latter mixes with extracellular fluid in approximately the ratio of one to one.

Some of the acid buffer solutions used in the tests were subsequently mixed, in the ratio of 1:1, with serum and with a fluid resembling extracellular fluid. The extracellular-like fluid was prepared by mixing one part of serum with six parts of isotonic sodium chloride solution, the resulting mixture having a protein content approximating that of extracellular fluid and a pH of 6.9.

The following alterations of pH after mixing were observed, each pH value being the mean from three experiments.

| BUFFER SOLUTION | ADDED SOLUTION PROPORTION 1:1 | pH OF BUFFER SOLUTION | pH AFTER ADMIXTURE |
|---|---|---|---|
| KCl-HCl buffer | Extracellular fluid | 1.1 | 1.5 |
| Acetate buffer | „ „ | 3.2 | 3.9 |
| „ „ | „ „ | 5.1 | 5.5 |
| „ „ | Serum | 3.2 | 4.1 |
| „ „ | „ | 5.1 | 5.9 |

No far-reaching conclusions can be drawn from these experiments, in which the tested fluids did not come into contact with the circulating blood and in which the bicarbonate-carbonate buffer was not in action. In the above-mentioned mixtures the hydrogen ion concentration of the buffer solutions was invariably reduced.

## CHAPTER 12 RELATIONSHIP OF INTENSITY TO DURATION OF PAIN

With the procedure employed, the pain responses to each test solution consisted of two factors: the pain intensity and the pain duration. Figures 10 to 15 (pages 39, 45, 52, 55) indicate that on the whole these two factors showed parallel variations with changes in potency of the pain-producing agent. Regression analysis of this relationship discloses for hypertonic, hypotonic, alkaline, acid, and potassium ion-containing solutions, a highly significant correlation between intensity and duration of pain ($p < 0.001$ in each instance).

From figure 16, in which the regression lines for the various solutions are collected, it is evident that the correlation varies substantially for the different types of solutions. The regression is greater for hypertonic than for acid solutions, the duration at a given intensity being approximately four times longer for a hypertonic than for an acid solution.

It follows that intensity and duration of pain differ in precision as criteria of the pain-producing effect of a test solution. The lower the *b* value, the more precisely does the pain intensity reflect the magnitude of the painful stimulus applied. For the types of solutions tested in this investigation the pain intensity was in all cases the more precise of the two criteria. This is evident from the *t* tests of the *b* lines for the correlation between painful stimulus and intensity or duration of pain: The *p* values were invariably lower for intensity than for duration.

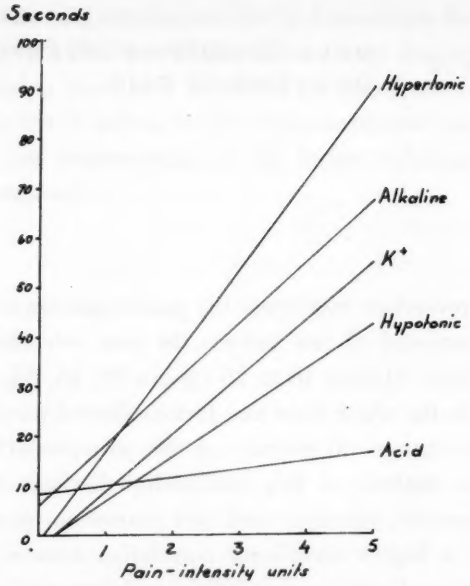



*Fig. 16* Regression lines for the relation of intensity to duration of pain with different types of pain-producing solutions. The line for hypertonic solutions is based on 180 injections of six solutions in 15 subjects ($b$ value 1.83); that for hypotonic solutions, on 240 injections of six solutions in 20 subjects ($b$ value 0.90); that for potassium-ion containing solutions, on 320 injections of eight solutions in 20 subjects ($b$ value 1.15); that for alkaline solutions, on 280 injections of seven solutions in 20 subjects ($b$ value 1.22); and that for acid solutions, on 280 injections of acetate buffer solutions in 20 subjects ($b$ value 0.17).

Pain intensity and pain duration seem to reflect different mechanisms. The intensity may be regarded as a criterion not only of the agent's pain-producing effect but of the subject's individual sensitivity. The duration presumably is more dependent on local peripheral conditions such as blood circulation, buffering capacity of the tissue, etc. In the discussion the main emphasis will be on the behavior of the pain intensity.

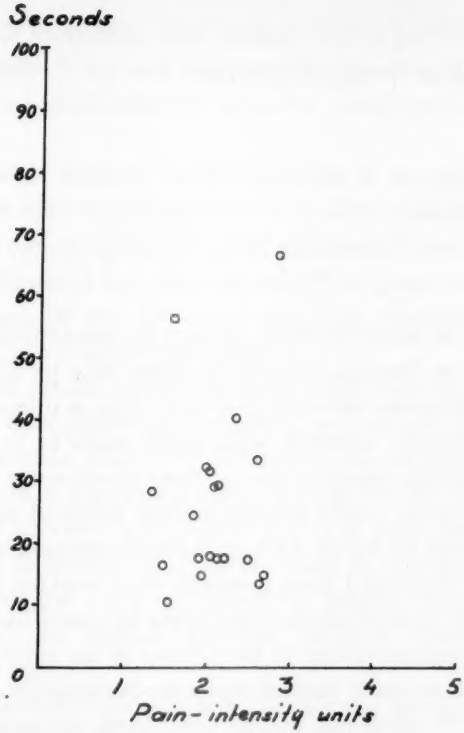



*Fig. 17* Diagram showing average pain intensity and duration in 20 subjects who had received identical injections of 48 different test solutions. Each point represents the mean of 96 injections in one subject.

On comparing the average intensity and duration of the pain responses in the individual subjects, it is found that those two factors are not correlated but vary quite independently of each other; i.e., a high as well as a low intensity may coincide with either a long or a short duration (figure 17). This behavior of the pain responses lends support to the view that pain intensity and pain duration are conditioned by different mechanisms.

## CHAPTER 13 | DISCUSSION

Pain can be experimentally elicited by mechanical, thermal, electrical or chemical stimuli. To these must be added all of the various means whereby pain may arise in disease.

No generally accepted explanation exists as to how such varying stimuli can give rise to identical subjective sensations. An explanation would emerge if these different pain mechanisms could be traced back to a single common mechanism. In this connection I have assumed, as a working hypothesis, that all the various mechanisms might be traced back to a chemical change occurring in the vicinity of the nerve terminals. This idea is based largely upon the investigations of *Lewis et al.* [53, 54] concerning muscle pain. Lewis assumed that such pain arises from the formation of some chemical substance (pain factor) in working anoxic muscle.

If such a substance exists, it should (1) be demonstrable in most tissues, particularly the skin, in normal or painful conditions; and (2) be pain-producing in concentrations equivalent to those occurring in the organism.

In the following the present experimental results will be considered on the basis of this hypothesis.

### Osmotic Pressure

Both depression and elevation of the osmotic pressure relative to that of the body give rise to pain, provided the alteration is

of sufficient magnitude. This finding verifies previous investigatory results. It has now been possible, however, to establish the range of pain-inducing pressures more precisely than before.

The osmotic pressure in the organism is normally fairly constant. In extreme disturbances of the fluid balance, depression of the osmotic pressure to 203 milliosmol/l and elevation to 375 milliosmol/l have been recorded[90]. A glance at tables 2 and 3 (pages 38 and 40) shows that such pressure changes do not give rise to appreciable pain.

Somewhat greater osmotic pressure shifts have been found in cases of local pathologic conditions, particularly inflammation and necrosis. *Ritter*[71, 72] determined the reduction of the freezing point in pus from a series of furuncles. He found that the osmotic pressure was usually 1.1—1.4 times the normal (340—434 milliosmol/l). In a few cases it amounted to 2.5 times the normal (775 milliosmol/l). Similar experimental elevation of the osmotic pressure, however, produced no appreciable pain.

With a moderate reduction of the osmotic pressure, injection of hypotonic solutions causes no pain; not until the pressure falls to 121 milliosmol/l or less does pain result, and thereafter it rises steeply. One plausible interpretation of this finding is that the cells in the skin, like the red blood corpuscles, are able in some degree to resist falls in osmotic pressure. Red blood cells are hemolyzed normally at an osmotic pressure of 146—166 milliosmol/l. An increase of pain does in fact coincide with reduction of the osmotic pressure below those values; hence it might be surmised that the pain arises when the cells are disrupted and their contents come into contact with the nerve terminals. This hypothesis would indicate that the cell content itself was pain-producing.

**Inorganic Ions**

Of various ions tested ($H_4N^+$, $Na^+$, $K^+$, $Ca^{++}$, $Mg^{++}$, $Cl^-$, $HCO_3^-$, $HPO_4^{--}$, and $SO_4^{--}$) only potassium induced appreciable pain. Table 12 shows the inorganic ion concentrations found in serum.

**Table 12** Serum content of inorganic ions. Figures in parenthesis are bibliographic reference numbers.

| ION | TEST CONCENTRATION mN | NORMAL SERUM CONCENTRATION mN | PATHOLOGIC VARIATION mN |
|---|---|---|---|
| $H_4N^+$ | 150 | 0.06-0.12 (56) | — |
| $Na^+$ | 150 | 137-148 (38) | 113-174 (38) |
| $K^+$ | 5—99 | 4-6 (38) | 2-15 (38) |
| $Ca^{++}$ | 113 | 4.5-5.5 (38) | 2-15 (38) |
| $Mg^{++}$ | 132 | 1.4-2.4 (56) | — |
| $Cl^-$ | 150 | 96-107 (38) | 28-169 (38) |
| $HCO_3^-$ | 175 | 22-30 (38) | 1-60 (38) |
| $HPO_4^{--}$ | 65 | 1.6-2.6 (56) | — |
| $SO_4^{--}$ | 66 | 0.7-1.2 (56) | — |

Though the concentrations tested were usually far higher than those in serum—in some instances, 1,000 times higher—they still caused no appreciable pain.

Quite different, however, was the behavior of potassium, whose pain-inducing properties have long been recognized[32]. In this investigation the pain elicited at a potassium ion concentration of 32 mN was significant even though of short duration and classed as less than slight. Indeed, even at the highest concentration tested (99 mN), the average pain intensity was less than moderate.

A potassium concentration of between 19 and 24 mN has

been found in normal skin[69, 84]. In psoriatic skin the potassium content is elevated to 37 mN[69]. A $K^+$concentration of 7—12 mN has been demonstrated in inflammatory exudate[60], and of 6—52 mN in pus[42], the content rising with the intensity of the inflammation. These values hardly support the view that the potassium ion concentration is that factor which elicits the pain in inflammation.

**Organic Metabolites**

Of the various metabolites tested (creatinine, creatine, sodium citrate, sodium lactate, sodium acetate, sodium succinate and sodium pyruvate), sodium citrate alone gave rise to pain. Scrutiny of table 13 reveals that the tested concentrations of these substances exceeded the normal serum concentrations by approximately 100—1,000 times.

**Table 13** Serum content of some organic metabolites. Figures in parenthesis are bibliographic reference numbers.

| SUBSTANCE | TEST CONCENTRATION mM | NORMAL SERUM CONCENTRATION mM | PATHOLOGIC VARIATION mM |
|---|---|---|---|
| Creatinine | 272 | 0.05—0.10 (56) | — |
| Creatine | 99 | Traces in muscle, 23-38 (36) | — |
| Sodium citrate | 108 | 0.10-0.14 (38, 56) | 0.05-0.52 (38) |
| Sodium lactate | 218 | 0.9-1.7 (56, 79) | 4.5-26.3 (10, 50, 79) |
| Sodium acetate | 151 | Traces | — |
| Sodium succinate | 101 | Traces | — |
| Sodium pyruvate | 153 | 0.03-0.15 (56, 79) | 0.03-0.85 (10, 21, 79) |

Thus I was unable to verify *Fleckenstein's*[26] observation that pyruvic acid, succinic acid and citric acid (neutralized isotonic solution) in concentrations of one-half to one-twentieth of that

tested here, give rise to appreciable pain. From my results it appears unlikely that these metabolites play a major role in the elicitation of pain.

**Histamine, Serotonin, and Acetylcholine**

In recent decades these substances have attracted considerable interest because of their high biologic activity. All three, moreover, have been ascribed pain-producing properties; indeed, in some investigations, even very low concentrations have been found to induce pain (e.g. histamine in a concentration of $10^{-18}$ g/g). My experiments confirm that these substances are pain-producing in concentrations of $10^{-4}$ to $10^{-3}$ g/ml but not below. The pain induced at those concentrations is, however, slight and of short duration.

**Table 14** Histamine, serotonin and acetylcholine content in various tissues.

| TISSUE | SUBSTANCE | CONCENTRATION G/ML | BIBLIOGRAPHIC REFERENCE NO. |
|---|---|---|---|
| Skin, rat | Histamine | $2.6\times10^{-5}$ | 31 |
| Skin, human | „ | $10^{-6}$-$10^{-5}$ | 94 |
| Small intestine, human | „ | $4\times10^{-5}$-$10^{-4}$ | 94 |
| Diverse tissues, animal | Serotonin | $10^{-7}$-$10^{-4}$ | 18, 19, 52 |
| Carcinoid tumor, human | „ | $6\times10^{-4}$-$2.5\times10^{-3}$ | 52 |
| Snake venom | „ | $3\times10^{-3}$ | 52 |
| Nerves, animal | Acetylcholine | $10^{-8}$-$10^{-6}$ | 24, 27 |
| Brain, animal | „ | $10^{-6}$ | 24 |
| Cerebrospinal fluid, human | „ | $10^{-6}$ | 80, 91 |
| Non-nervous tissues | „ | Very low or indeterminable | 24, 57 |

The concentrations of these substances in different tissues are set forth in table 14, from which it will be observed that the levels required for inducing pain are 100—1,000 times higher than those concentrations found in the tissue. This fact militates against the view that any one of these substances acts as a chemical mediator in the elicitation of pain. Yet it is conceivable that, even if a tissue contains a low total concentration of the substance, considerably higher and perhaps painful concentrations may arise locally near a surface or in a membrane. To some extent, therefore, the question must be left open.

**Hydrogen Ion Concentration**

Reduction of the hydrogen ion concentration does not induce significant pain until the concentration has fallen to a level well below that normal for the body, and the pain subsequently rises in moderate degree with a rising hydroxyl ion concentration. Since the organism is not known to be subject to any major deviations of pH in this direction, such a deviation cannot be regarded as a cause of pain.

Elevation of the hydrogen ion concentration gave rise to significant pain at pH 6.2. The pain thereafter increased in proportion to the hydrogen ion concentration, reaching at pH 3.2 the highest average intensity values recorded. The same behavior was observed in both runs of experiments with acid buffer solutions. A buffer solution of pH 1.1 was included in one of these runs. Although an accentuation of the pain response might have been expected here, the response was, in fact, largely the same for pH 3.2 as for pH 1.1. There are several possible ways of accounting for this. I am inclined to believe that the painful stimulus to the receptors is already maximal at pH 3.2, so that a further rise in the hydrogen ion concentration does not increase the pain intensity but simply

prolongs the duration. This seems to be evidenced by the fact that at pH 3.2 the pain duration was 37 seconds, while at pH 1.1 it was 58 seconds.

In evaluating pain responses to solutions with elevated hydrogen ion concentrations it is, as with other solutions, worthwhile to compare the hydrogen ion concentrations demonstrable in the organism. In this connection see table 15.

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**Table 15**

| TISSUE OR ORGAN | pH | BIBLIOGRAPHIC REFERENCE NO. |
|---|---|---|
| Arterial blood, normal, human | 7.35-7.45 | 56 |
| Venous blood, chronic nephritis, human | 7.05 | 44 |
| Subcutaneous tissue, normal, human | 7.3 | 70 |
| Synovia, normal, human | 7.35 | 78 |
| Cancellous bone, human | 7.6 | 78 |
| Muscle, normal, human | 7.45 | 78 |
| Connective tissue, normal, human | 7.45 | 78 |
| Connective tissue, muscle, inflamed, human | 5.7-7.2 | 78 |
| Tumor tissue, painful, human | 5.5-6.1 | 70 |
| Tumor tissue, painless, human | 7.6-8.8 | 70 |
| Pus from abscess, human | 5.5-6.0 | 39 |
| Liver, normal, dog | 7.10 | 6 |
| Liver, circulation arrested, dog | 6.30 | 6 |
| Muscle, resting, guinea pig | 7.3-7.4 | 28 |
| Muscle, working, guinea pig | 6.5 | 28 |
| Muscle, chemically inflamed, guinea pig | 6.0-6.5 | 28 |

| | | |
|---|---|---|
| Muscle, postinflammatory necrosis, guinea pig | 5.0 | 28 |
| Kidney, normal, guinea pig | 6.7-6.9 | 40 |
| Kidney, anoxic, guinea pig | 6.0 | 40 |
| Muscle, normal, guinea pig | 7.0 | 61 |
| Muscle, exhausted, guinea pig | 5.8 | 61 |
| Inflammatory exudate, dog | 6.5-7.4 | 59, 60 |
| Fracture hematoma, dog | 4.7 | 65 |
| Abscess, subcutaneous, dog | 5.2-6.1 | 68 |
| *Intracellular pH* | | |
| Kidney, human | 5.0-7.1 | 17 |
| Gastric mucosa, human | 6.5-7.0 | 17 |
| Liver, human | 5.0-7.1 | 17 |
| Pancreas, human | 5.0-7.2 | 17 |
| Muscle, normal, human and animal | 7.1 | 17 |
| Muscle, exhausted, animal | 6.3 | 17 |
| Muscle, exhausted and rigid, animal | 5.0-7.0 | 17 |
| Muscle, normal, dog | 6.6-7.1 | 92 |
| Muscle, normal, cat | 6.9 | 93 |

Scrutiny of the tabulated figures indicates that the pH may vary substantially according to e.g. the type and condition of the tissue, etc. Interstitially and in healthy tissue the pH is usually in the vicinity of the normal blood value. The intracellular pH is in general lower, with values down to 5.0. It may also fall to as low as 5.0 in damaged, anoxic and, in the case of muscle, exhausted tissue. With inflammation the tissue pH descends to values around 5.0. These figures indicate that elevation of the hydrogen ion concentration to a level which, experimentally, is pain producing (pH 6.2) occurs both intracellularly under normal conditions, and in anoxic, damaged, or inflamed tissue.

The hydrogen ion concentration in these conditions may sometimes be as much as 16 times higher (pH 5.0). A buffer solution of pH 5.1 evokes moderate to severe pain (intensity 3.34 units). Severe and even very severe pain (4.0 and 5.0 units) may of course attend inflammation and injuries, but pH values equalling those which experimentally give rise to such high pain intensities (pH 4.1 — 3.6 — 3.2) have not been demonstrable in fluids or tissues either under normal or pathologic conditions.

Can an elevated hydrogen ion concentration, in these circumstances, be regarded as a cause of pain of all intensities? In the case of other tested substances such as the potassium ion or histamine, it is known that — disregarding immediate dilution by extracellular fluid — the concentration injected will persist in the tissue for a time. The concentration thereafter decreases gradually via diffusion, in which connection the blood circulation is an important factor in removal of the ions present in excessive concentrations.

In the case of hydrogen ions, the situation is different. By means of various buffering mechanisms the hydrogen ion concentration in the blood and the body is maintained at a relatively constant level. In the blood, hemoglobin, serum protein, bicarbonate-carbonate and monophosphate-diphosphate are the principal buffer systems[81]. In principle the same buffering mechanisms exist, via the blood circulation, in the tissues. It may be assumed, furthermore, that in most tissues buffering takes place via exchange of intracellular potassium for hydrogen ions[25, 82].

If, therefore, a buffer solution of pH 3.2 is injected into a tissue, the relevant hydrogen ion concentration can scarcely be expected to exist for more than a very short time; via the buffering mechanisms of the body, it will be swiftly lowered towards the normal level. The velocity of this fall and the time

required for normalization of the tissue pH, are not known; and indeed they would be very difficult to elucidate with available apparatus. True, microelectrodes have been developed for pH determination, but they are not fine enough for intracutaneous use in measuring pH changes during and after the production of a wheal.

*Björn*[9] determined the pH in rabbit subcutis following subcutaneous injections of acid solutions of local anesthetics. He found that 30—45 minutes elapsed before the pH was normalized. At the initial determination a few minutes after the injection, the pH had fallen by an average of one unit.

My own experiments with mixtures of buffer solutions and serum or extracellular-like fluid, pointed in the same direction. Normalization of the pH may be expected to proceed far more swiftly in the highly vascular cutis. In view of these facts, therefore, the pH values of the buffer solutions were scarcely equivalent to the hydrogen ion concentrations actually being tested; namely, those in the skin immediately after the injection. The concentrations actually tested were invariably lower — probably far lower and rapidly falling. Some support for this assumption lies in the fact that the pain evoked by acid solutions — in contrast to that produced by other solutions — is dissipated with the greatest rapidity; even at the highest intensities it has vanished after an average of 19 seconds.

When, on the other hand, a pH of 6.2 is found in inflammatory exudate, one knows that the value remains constant over a long period. In a buffer solution of pH 6.2, however, the hydrogen ion concentration will probably be lowered towards normal very swiftly. This difference must be borne in mind when comparing pH values determined in tissues with those tested by means of buffer solutions.

In my view, the fact that severe and very severe pain is not, on the average, produced by buffer solutions until their hydro-

gen ion concentrations exceed those demonstrable in the body, does not necessarily conflict with the possibility that an elevated hydrogen ion concentration in the organism may be a cause even of severe and very severe pain.

In view of the above considerations the hydrogen ion concentration may be described as the only one of the factors investigated which satisfies the aforementioned desiderata for a chemical mediator of pain; namely, that (1) it must be demonstrable in most tissues under normal or painful conditions, and (2) it must be pain-producing in the concentrations which may occur in the organism. On the basis of this statement it may be of interest to discuss in greater detail the behavior, both actual and hypothetical, of the hydrogen ion concentration in various painful conditions, cutaneous and otherwise.

Pain can be induced experimentally not only by electrical means but by chemical, thermal and mechanical stimuli. What are the conceivable ways in which these might be traced back to an altered hydrogen ion concentration? No determinations of the cutaneous pH associated with these forms of stimuli have been reported. Hypothesizing first a low intracellular pH (6 to 5), it would seem reasonable to assume that chemical, thermal, and mechanical stimuli could disrupt the cells to such an extent that their acid contents would come into contact with the nerve terminals. Since, moreover, the cellular content is rich in protein and hence has a high buffering capacity, its pH value may be assumed to persist for a relatively long period. This view is supported in some measure by the fact that the pain evoked by injection of distilled water — which must be assumed to have caused disruption of the cells and release of their contents — was among the most severe recorded. The pain was equal in intensity to that produced by the most acid of the buffer solutions, and its duration was twice as long.

There are numerous painful conditions and diseases in

which an elevated hydrogen ion concentration has been observed. A type example is the anoxic pain associated with e.g. angina pectoris and intermittent claudication, conditions in which elevation of the hydrogen ion concentration is known to occur via a largely glycolytic intracellular metabolism[20]. A low pH has been demonstrated in anoxic skeletal muscle[28], anoxic heart muscle[20], anoxic kidney tissue[40], and anoxic liver tissue[6]. A reduced pH also results from the formation of acid metabolites during heavy muscle exercise with a normal blood circulation[28, 61]. — An increased hydrogen ion concentration with pH 4.7 has been demonstrated in fracture hematomas[65].

*Moore*[62] was able to elicit in animals, by intra-arterial injection of acid buffer solutions, an increased reflex activity as well as vocal manifestations as indications of pain.

The pain associated with gastric or duodenal ulcer has been studied by several authors, who conclude that such pain is attributable to acidity of the gastric contents and subsides with alkalization of the latter[12, 63, 64].

A low pH has been found in tissue from painful tumors, in contrast to painless tumors where the pH has been normal[70].

An elevated hydrogen ion concentration has been recorded in inflamed tissue, inflammatory exudate, and pus[28, 59, 60, 68, 78].

*Gaza*[30] found that injection of alkaline solutions into painful abscesses and furuncles wherein the pH was low, eliminated the pain. He also demonstrated that tuberculous abscesses (which usually are painless) had a normal hydrogen ion concentration and that injection of acid buffer solutions into them gave rise to pain.

Although the question has not, of course, been definitely elucidated, all these investigations nevertheless suggest that the hydrogen ion concentration may be the chemical mediator serving to trigger the pain stimulus in the nerve terminals of different tissues.

Concerning the two aims of this investigation mentioned in the introduction, I would conclude that

(1) The present method of inducing and measuring experimental skin pain permits comparison of the pain-producing effects of eight concurrently tested aqueous solutions. Following statistical analysis of the data obtained, it is possible to determine the significance of observed pain and also of observed differences in the painful effects of the various solutions.

(2) This uniform method was employed to test a number of those substances which occur in the organism and are thought to be associated with pain. Various substances did in fact evoke pain, but only the hydrogen ion had pain-producing properties of such magnitude that it might reasonably be regarded as a chemical mediator of skin pain.

## CHAPTER 14 SUMMARY

### Review of the Literature

The relatively few previous experimental investigations on chemically induced skin pain are outlined, as are various chemical methods of inducing skin pain. Procedures used for estimation of the intensity of experimental pain are surveyed.

### Experimental Method

The author's experimental method is described with reference to injections of various test solutions by a special jet injector. The injections were given intra- and subcutaneously in volunteers, eight different solutions being tested concurrently in 15—21 subjects. The estimated pain intensity was graded according to an ordinal scale ranging from 0 to 5 in half units, and the pain duration was recorded in seconds.

### Statistical Analysis

The statistical data and methods are reported.

### Basal Pain Associated with the Method

Injection of a non-pain-producing solution (isotonic sodium chloride) occasionally gave rise to slight to moderate pain. The average pain response to the above solution was called

the basal pain associated with the method and was attributed to the fact that the fluid jet, in rare cases, injures the tissue and causes "traumatic pain". Only if the average pain response to a test solution differed significantly from this basal pain was the relevant solution considered pain-producing.

**Standard Deviation and Reproducibility of the Method**

The standard deviation of the method was determined by repeated injections of the same solution, and its reproducibility by repeated injections of the same test solutions on different occasions. The standard deviation is reported. The reproducibility was satisfactory.

**Test Solutions**

A description is given of the composition and properties of the test solutions employed.

**Osmotic Pressure**

Solutions with osmotic pressures two to six times higher than that normal for the organism gave rise to pain commensurate with the elevation of pressure. Solutions with reduced osmotic pressures did not evoke pain until the level fell to 0.4 times the body normal. The pain then increased proportionately with the reduction of osmotic pressure, reaching with distilled water an intensity which was one of the highest recorded in the entire investigation.

**Inorganic Ions**

The cations and anions normally occurring in the blood were investigated. With the exception of potassium, they were not pain-producing in isotonic concentrations. The potassium ion

induced pain proportional to the rise of concentration over and above the normal level in the blood. At the highest concentration tested — approximately twenty times that in plasma — the pain was, however, of moderate intensity.

**Organic Metabolites**

Creatinine, creatine, and the sodium salts of citric acid, lactic acid, acetic acid, succinic acid and pyruvic acid were tested in isotonic concentrations and as neutral salts. They produced little or no pain.

**Histamine, Serotonin and Acetylcholine**

Each of these substances caused slight pain in concentrations of $10^{-4}$ to $10^{-3}$ g/ml, but not in weaker concentrations. None of them gave rise to pruritus.

**Hydrogen Ion Concentration**

Alkaline buffer solutions evoked no significant pain until the pH values reached 9.1 and 10.1. Acid buffer solutions produced significant pain at pH 6.2. The pain then rose in proportion to the hydrogen ion concentration, reaching at pH 3.2 the highest average intensity values recorded in the investigation. The pain duration was very short for all acid solutions.

**Relationship of Intensity to Duration of Pain**

Each pain response is made up of an intensity factor and a duration factor. With changes in the magnitude of any given painful stimulus, these two factors showed a largely parallel variation. Analysis of their interrelationship showed a significant correlation. This varied with different types of solutions,

though for all types both factors served as criteria of the pain magnitude.

**Discussion**

The pain-inducing properties of the test solutions are discussed. The pain-producing concentrations of the various factors are compared with those concentrations which may occur in the organism under normal and pathologic conditions. It is concluded that of the factors investigated the hydrogen ion concentration is the only one which, at the levels which may be found in the skin, gives rise to pain. Moreover, there is substantial evidence to indicate that it may act as a mediator of pain in numerous and diverse painful conditions.

# ZUSAMMENFASSUNG


**Literaturübersicht**

Es wird über die bisherigen, verhältnismässig spärlichen Versuche mit experimentellen Hautschmerzen berichtet, ebenso über verschiedene Verfahren zur Erzeugung chemischen Hautschmerzes. Methoden für die Schätzung des experimentellen Schmerzes werden besprochen.

**Versuchsmethodik**

Die Versuchsmethodik des Verfassers wird geschildert. Bei ihr werden verschiedene Testlösungen mit einem Düseninjektor eingespritzt. Diese Injektionen werden intra- und subkutan freiwilligen Versuchspersonen verabfolgt, wobei acht verschiedene Testlösungen nebeneinander an 15 bis 21 Personen geprüft werden. Die Stärke des Schmerzes wird in fünf Schmerzstärkeeinheiten mit halben Intervallen abgeschätzt und die Schmerzdauer in Sekunden gemessen.

**Zahlenauswertung und Statistik**

Es wird über die Auswertung der Zahlen und über die statistischen Verfahren berichtet.

**Grundschmerz der Methode**

Die Injektion einer nicht schmerzenden Lösung (isotone Kochsalzlösung) erzeugt in einzelnen Fällen schwachen bis mässigen Schmerz. Die durchschnittliche Wirkung dieser Lösung wird

als Grundschmerz der Methode bezeichnet; sie ist wahrscheinlich darauf zurückzuführen, dass der Flüssigkeitsstrahl in einzelnen Fällen das Gewebe beschädigt und einen „Gewebeschadenschmerz" verursacht. Nur wenn sich der durchschnittliche Schmerz, der durch eine Testlösung hervorgerufen wird, signifikant von jenem Grundschmerz unterscheidet, gilt die Testlösung als Schmerz-Erzeuger.

**Streuung und Reproduzierbarkeit der Methode**

Durch wiederholte Einspritzungen der gleichen Lösung wurde die Streuung der Methode ermittelt, und durch wiederholtes Einspritzen der gleichen Testlösung bei verschiedenen Gelegenheiten wurde die Reproduzierbarkeit nachgeprüft. Es wird über die Streuung berichtet. Die Reproduzierbarkeit ist gut.

**Testlösungen**

Es wird über die Zusammensetzung und die Eigenschaften der angewandten Testlösungen berichtet.

**Osmotischer Druck**

Lösungen mit einem auf das Doppelte bis Sechsfache des im Körper vorhandenen Normalwertes erhöhten osmotischen Druck erzeugen einen Schmerz, der zur Erhöhung des Druckes proportional ist, während Lösungen mit verringertem osmotischem Druck keinen Schmerz verursachen, bevor die Erniedrigung das 0,4-fache des im Körper normalen osmotischen Druckes erreicht hat. Dann verstärkt sich der Schmerz im Verhältnis zur Erniedrigung des osmotischen Druckes, bis er beim destillierten Wasser die stärksten Schmerzstufen erreicht, die bei der Untersuchung beobachtet worden sind.

**Anorganische Ionen**

Die gewöhnlichen im Blut vorkommenden Kationen und Anionen wurden untersucht. Mit Ausnahme des Kaliums verursachen sie in isotonen Konzentrationen keinen Schmerz. Das Kaliumion erzeugt einen Schmerz, der zur Konzentrationserhöhung über den normalen Gehalt des Blutes hinaus proportional ist. Bei der höchsten geprüften Konzentration, die etwa das Zwanzigfache der Konzentration des Serums betrug, ist jedoch der erzeugte Schmerz von mässiger Stärke.

**Anorganische Metabolite**

Kreatinin, Kreatin und die Natriumsalze der Zitronensäure, Milchsäure, Essigsäure, Bernsteinsäure und Pyrotraubensäure wurden in isotonen Konzentrationen und als neutrale Salze geprüft. Sie erzeugen keinen oder nur unbedeutenden Schmerz.

**Histamin, Serotonin, Acetylcholin**

Diese Stoffe erzeugen alle einen schwachen Schmerz in der Konzentration $10^{-4}$ bis $10^{-3}$ g/ml, aber nicht in schwächeren Konzentrationen. Keiner dieser Stoffe verursacht Jucken.

**Wasserstoffionen**

Pufferlösungen alkalischer Reaktion erzeugen einen signifikanten Schmerz erst bei pH-Werten zwischen 9,1 und 10,1. Bei Pufferlösungen saurer Reaktion entsteht ein signifikanter Schmerz bei pH = 6,2. Der Schmerz verstärkt sich dann proportional zur Wasserstoffionenkonzentration, bis er bei pH = 3,2 die höchsten durchschnittlichen Schmerzstärkewerte erreichte, die bei der Untersuchung beobachtet worden sind. Die Dauer des Schmerzes war bei allen sauren Lösungen sehr kurz.

**Beziehung zwischen Stärke und Dauer des Schmerzes**

Nach jeder Injektion kann die Stärke und die Dauer des Schmerzes registriert werden. Bei Veränderung der Stärke des Schmerzreizes variieren diese beiden Faktoren nahezu parallel. Die Analyse ihres gegenseitigen Verhältnisses zeigt eine signifikante Regression. Diese ist bei verschiedenen Typen von Lösungen verschieden, aber bei sämtlichen Lösungstypen sind die Stärke und die Dauer ein Mass für die Grösse des erzeugten Schmerzes.

**Erörterung**

Die schmerzerzeugenden Eigenschaften der einzelnen Lösungen werden erörtert. Dabei werden die den Schmerz verursachenden Konzentrationen der verschiedenen Faktoren mit denjenigen Konzentrationen verglichen, die im Körper unter normalen und pathologischen Verhältnissen auftreten können. Als Ergebnis dieser Erörterungen zieht der Verfasser den Schluss, dass die Wasserstoffionenkonzentration der einzige untersuchte Faktor ist, der Schmerzen bei den Konzentrationen verursacht, die in der Haut entstehen können, und dass viele Gründe dafür sprechen, dass die Wasserstoffionenkonzentration bei einer Menge verschiedenartiger schmerzhafter Zustände ein Vermittler des Schmerzes sein könnte.

# RÉSUMÉ


**Aperçu de littérature**

Précédemment, comparativement peu de rapports avaient été faits sur des épreuves de douleur de peau experimentale par suite d'une réaction chimique, ainsi que sur les méthodes provoquant une telle douleur. Les méthodes d'évaluation de la force de la douleur expérimentale sont passées en revue.

**Méthode d'expérimentation**

Il est rendu compte de la méthode d'expérimentation de l'auteur. Différentes solutions de test sont injectées à l'aide d'un injecteur à jet direct. Les injections — intracutanées ou souscutanées — sont faites à des personnes bénévoles, huit différentes solutions de test étant essayées sur 15 à 21 sujets. La force de la douleur est évaluée d'après un barème de cinq unités, avec des demi-intervalles, et la durée, en secondes.

**Interprétation des chiffres et statistique**

Il est rendu compte de l'interprétation des chiffres et des méthodes statistiques.

**Douleur basale de la méthode**

L'injection d'une solution indolore (solution isotonique NaCl) provoque dans des cas isolés une douleur faible ou modérée.

La douleur moyenne causée par une telle solution est nommée dans la méthode "douleur basale", estimée provenir de ce que, dans certains cas, le jet du liquide endommage les tissus, occasionnant ainsi une "douleur de tissus endommagés". Ce n'est que dans le cas où la douleur moyenne provenant d'une solution de test s'écarte considérablement de la dite douleur basale que la solution est considérée être douloureuse.

**Dispersion et stabilité de la méthode**

Par des injections répétées d'une même solution a été étudiée la dispersion de la méthode, et par la répétition d'injections d'une même solution dans des conditions différentes, sa stabilité. La stabilité est bonne.

**Solutions de test**

Les solutions à pression osmotique 2 à 6 fois plus grande que la pression normale du corps provoquent une douleur en proportion de l'augmentation de la pression. Les solutions à pression osmotique basse ne provoquent pas de douleur tant que la pression n'est pas descendue jusqu'à 0,4 de la pression osmotique normale du corps. La douleur augmente ensuite en proportion de la diminution de la pression osmotique pour atteindre, après une injection d'eau distillée, un degré de douleur des plus forts enregistrés à l'examen.

**Ions inorganiques**

Une étude a été faite des cations et anions ordinaires se trouvant dans le sang. A l'exception du potassium, ils ne provoquent pas de douleur en concentrations isotoniques. L'ion de potassium cause une douleur proportionnelle à l'augmentation de la concentration excédant la teneur normale du sang. A la

plus haute concentration éprouvée, environ 20 fois celle du plasma, la douleur provoquée est, cependant, d'une force modérée.

**Métabolisants organiques**

La créatinine, la créatine et les sels de sodium des acides citrique, lactique, acétique, succinique et pyroracémique ont été expérimentés en des concentrations isotoniques et comme sels neutres. Ils ne provoquent aucune douleur, sinon une douleur insignifiante.

**Histamine, sérotonine, acétylcholine**

Ces solutions provoquent toutes une faible douleur en des concentrations de $10^{-4}$ à $10^{-3}$ g/ml, mais non en des concentrations plus faibles. Aucune de ces substances ne provoque de prurit.

**Ions hydrogènes**

Les solutions neutralisantes à réaction alcaline provoquent une douleur significative seulement à une valeur pH entre 9,1 et 10,1. Les solutions neutralisantes à réaction acide provoquent une douleur significative à pH 6,2. La douleur augmente ensuite en proportion de la concentration des ions hydrogènes pour atteindre à pH 3,2 des valeurs moyennes de douleur les plus élevées enregistrées à l'examen. La durée de la douleur pour toutes les solutions acides est très courte.

**Relation entre la force et la durée de douleur**

A chaque injection sont obtenues deux réponses, dont l'une sur la force de la douleur, et l'autre, sur sa durée. A la modification de la force d'irritation douloureuse, les deux réponses

varient d'habitude parallèlement. L'analyse de leur relation réciproque montre une régression significative. Celle-ci est différente pour différents types de solutions, mais pour tous ces types, les deux réponses expriment la mesure de la force de douleur provoquée.

**Discussion**

Sont discutées les propriétés douloureuses des différentes solutions de test. Les concentrations douloureuses des différents facteurs y sont comparées avec les concentrations pouvant se produire dans le corps dans des conditions normales ou pathologiques. Comme résultat de cette discussion, l'auteur tire la conclusion que la concentration d'ions hydrogènes est le seul facteur étudié qui provoque la douleur parmi les concentrations pouvant se produire dans la peau, et qu'il y a beaucoup de raisons parlant en faveur de ce qu'une concentration d'ions hydrogènes pourrait être un agent de douleurs dans nombre de différents états de souffrance.

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